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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 :
C07D 475/02, 473/04, 513/04, 239/96, 217/24, 401/14, A61K 31/505, 31/52 // (C07D 513/04, 285:00, 239:00)

A1

(11) International Publication Number: WO 00/00491

(43) International Publication Date: 6 January 2000 (06.01.00)

(21) International Application Number: PCT/US99/14320

(22) International Filing Date: 24 June 1999 (24.06.99)

(30) Priority Data:
09/107,026 29 June 1998 (29.06.98) US

(71) Applicant: THE REGENTS OF THE UNIVERSITY OF CALIFORNIA [US/US]; 12th floor, 1111 Franklin Street, Oakland, CA 94607-5200 (US).

(72) Inventors: CARSON, Dennis, A.; 1482 Vista Del Oceano, Del Mar, CA 92014 (US). COTTOM, Howard, B.; 1890 Lenpee Drive, Escondido, CA 92025 (US). CHAO, Qi; Apartment 32, 4158 Decoro Street, San Diego, CA 92122 (US).

(74) Agent: BERLINER, Robert; Fulbright & Jaworski L.L.P., 29th Floor, 865 South Figueroa Street, Los Angeles, CA 90017-2576 (US).

(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: BIFUNCTIONAL ANTAGONISTS OF CYTOKINE-SENSITIVE PROTEIN KINASE ACTIVATION CASCADES AND METHODS FOR USE AS ANTI-INFLAMMATORY AGENTS

(57) Abstract

Bifunctional activation cascade antagonist compounds inhibit inflammatory responses associated with TNF-α and fibroblast proliferation *in vivo* and *in vitro* by blocking activation of both the c-Jun and p38 kinases. The compounds of the invention possess greater than additive activity as compared to the individual constituents of each compound. One such constituent blocks activation of c-Jun kinase while the other blocks activation of p38 kinase, in a non-overlapping manner. The compounds of the invention neither appreciably inhibit the activity of cAMP phosphodiesterase nor the hydrolysis of phosphatidic acid, and are neither cytotoxic nor cytostatic. Methods for the use of the novel compounds as anti-imflammatory agents are also described. The methods are expected to be of use in reducing or protecting a vertebrate host against inflammatory responses (for example, after angioplasty), in limiting fibrosis (for example, of the liver in cirrhosis), in inhibiting cell senescence, cell apoptosis and UV induced cutaneous immune suppression.

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PATENT

BIFUNCTIONAL ANTAGONISTS OF CYTOKINE-SENSITIVE PROTEIN KINASE ACTIVATION CASCADES AND METHODS FOR USE AS ANTI-INFLAMMATORY AGENTS

RELATED PATENT APPLICATIONS

This application is a continuation-in-part of US Patent Application Serial Number 08/858,778 filed May 19, 1997 (now pending), which is in turn a continuation-in-part of US Patent Application Serial Number 08/367,102, filed December 29, 1994 (now pending), which is in turn a continuation-in-part of US Patent Application Serial No. 08/482,551, filed June 7, 1995 (now allowed).

STATEMENT OF GOVERNMENT SUPPORT

This invention was made with government support under Grant No. GM-23200 awarded by the National Institutes of Health. The government may have certain rights in the invention.

BACKGROUND FOR THE INVENTION

1. *Field of the Invention*

The invention relates to antagonists of cytokine-sensitive, mitogen-activated protein kinase activation and their use as anti-inflammatory agents.

2. *History of the Related Art*

Two of the MAPK (mitogen activated protein kinase) signalling cascades are initiated by proinflammatory cytokines (PICs) and other stress factors, such as ultraviolet light, ionizing radiation, tissue trauma, cytotoxic agents (e.g., anisomycin and arsentine), heat shock and ceramide. These "PIC sensitive" signalling cascades lead to activation of the c-Jun N-terminal protein kinase (JNK; also known as SAPK, for "stress-activated protein kinase") and a newly discovered protein kinase, p38 (also known as CSBP, for "cytokine suppressive anti-inflammatory drug binding protein").

Activation of JNK and p38 is related to the onset and maintainence of acute and chronic inflammatory conditions such as localized fibrosis, cystic fibrosis, ultraviolet light induced cutaneous immune suppression, cell senescence and apoptosis, gastrointestinal inflammation (e.g., inflammatory bowel disease) and pulmonary inflammation (e.g., chronic bronchitis and asthma). Inhibition of PIC synthesis and/or release by stressed cells can reduce MAPK activation-related inflammation by avoiding initiation of the PIC sensitive MAPK signalling cascades.

Different anti-inflammatory agents approach this goal in different ways. For example, pentoxifylline suppresses synthesis of PICs by inhibiting cAMP phosphodiesterase activity. Corticosteroids inhibit transcription of PICs. Cytokine suppressive anti-inflammatory drugs (CSAIDs) are believed to inhibit PIC synthesis by binding a MAPK required for cytokine mRNA translation; these compounds selectively interfere with activation of the p38 signaling cascade. None of the drugs are completely effective in suppressing PIC synthesis or release and many have side-effects that are use-limiting.

SUMMARY OF THE INVENTION

The invention provides compounds which are bifunctional in that they block PIC initiation of both of the PIC sensitive MAPK activation cascades. In particular, the invention combines a novel antagonist of the JNK activation cascade with another PIC inhibitor, most preferably an antagonist of the p38 activation cascade. The components of the resulting "bifunctional activation cascade

antagonists" act synergistically to antagonize PIC-sensitive MAPK activation to a greater extent than could be achieved by either compound alone.

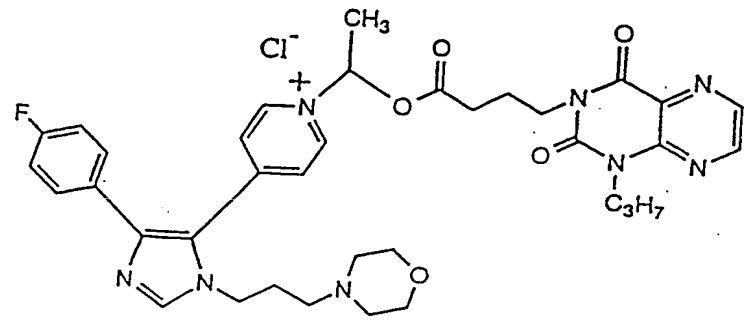
In particular, the bifunctional activation cascade antagonists of the invention inhibit up to 100% of PIC release by stressed cells. In constrast, 70-80% PIC inhibition is the maximum level achievable by known PIC-sensitive MAPK antagonists. Advantageously, bifunctional activation cascade antagonists do not pose the risk of side effects associated with certain anti-inflammatory agents, such as the sleeplessness and anxiety induced by methylxanthine-based cAMP phosphodiesterase inhibitors (e.g., pentoxifylline). Further, the potency of the inventive compounds permits their use at lower dosages than are required by use of their constituents alone.

The JNK activation cascade antagonist constituent of the inventive bifunctional activation cascade antagonists consists of heterocyclic molecules with biologically active side chains; specifically, purine, pteridine, thiadiazolopyrimidine, quinalozine and isoquinolone based compounds and water-soluble morpholinoethyl esters thereof. Such compounds and methods for their synthesis are described in detail in commonly owned US Patent Application Serial Nos.08/858,778, 08/367,102 and 08/482,551, the disclosures of which are incorporated herein as though set forth in full. The p38 activation cascade antagonist constituents are any compound which interferes with the initiation or completion of the p38 activation cascade. In one aspect of the invention, the p38 activation cascade antagonist is a pyridylimidazole. An especially useful class of pyridylimidazoles for use in the invention are the CSAIDs.

The JNK and p38 activation cascade antagonist constituents of the bifunctional activation cascade antagonists are preferably conjugated together. Thus, in one aspect of the invention, the constituents of the bifunctional activation cascade antagonists are conjugated to one another via a bond which is severable *in vivo*, such as ester, amide or azo linkages. In another aspect of the invention, unconjuated JNK and p38 activation cascade antagonist constituents are mixed in a pharmaceutically acceptable carrier. In yet another aspect of the invention, conjugated or unconjugated

JNK and p38 constituents of the bifunctional activation cascade antagonists are combined with a delivery vehicle, such as a colloidal dispersion system.

The invention further encompasses the following conjugated bifunctional activation cascade antagonists:



wherein n is any number of carbon atoms from 1 to 7, O or N;

R_1, if present, is H, an alkyl, a cyclic alkyl, a heterocyclic alkyl, alkenyl, or aralkyl having less than 7 carbon atoms;

R_6 and R_7 are H, OH or OR_1, in any combination;

Z is N or C;

X, where Z is C, is H, halogen, N_3, NO, NH_2, NHR_1, $N(R_1)2$ or COR_1; and,

A is H, halogen, N_3, NO, NH_2, NHR_1, $N(R_1)2$ or COR_1.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGURE 1 is a bar graph depicting inhibition of cell growth arrest in 3T3 fibroblasts according to the invention after growth arrest was induced through deprivation of the cells of serum. The cells were incubated and grown to 90% confluence in serum. The medium was then removed and replaced with serum-free medium. To assess the effect of an inventive compound (no. 37, a pteridine) on cell senescence in the presence of ceramide, aliquots of the cells were incubated with different concentrations of each. Concentrations of compound no. 37 are indicated by the insert legend while concentrations of ceramide are indicated along the x axis. Inhibitory effects were assessed as a measure of DNA synthesis; [^{3}H] thymidine incorporation detection is indicated along the y axis.

FIGURE 2 is a bar graph depicting inhibition of cell apoptosis in human (Jurkat) T lymphocytes according to the invention. The inhibitory activity of two inventive compounds (nos. 37 and 6 (a purine)) was tested in comparison to like activity of pentoxyfilline and a control compound, Ro 20-1724. Activation of the sphingomyelin signal transduction pathway was stimulated by incubation of the cells with an anti-FAS monoclonal antibody (which binds CD95, a cell surface receptor which triggers cell apoptosis). Percent inhibition was measured as a function of the number of cells which excluded vital dye erythrosin B. Percent inhibition is indicated along the y axis while the concentration of compounds tested is indicated along the x axis.

FIGURE 3 is a bar graph depicting inhibition of activity on the part of CaPK in Jurkat cells according to the invention. The inhibitory activity of a compound of the invention (no. 37) was tested in the presence of either ceramide or anti-FAS. Inhibition of CaPK activity was measured as a function of phosphorylation and detected by autoradiography. The compounds the cells were incubated in are indicated along the y axis while the percent control (i.e., inhibition of CaPK) is indicated along the x axis. Shorter bars indicate greater relative inhibition.

FIGURES 4 (a) through **(g)** are copies of spectrographs indicative of absorbance of inventive compounds no. 37, no. 6, no. 37 in combination with no. 6, oxo variants of nos. 37 and 6, as well as, for comparison, PABA (p-amino benzoic acid, a common sunscreen additive) and isoquinolone. The inventive compounds absorbed through most of the UVB wavelength, while a mixture of compound nos. 37 and 6 absorbed throughout the UVB wavelength.

FIGURES 5(a) and **(b)** depict, respectively, the results of an enzyme-linked immunosorbent assay (ELISA) for TNF-α production by bacterial lipopolysaccharide (endotoxin) stimulated human monocytes incubated with the compounds of the invention and a control compound (Ro-1724, that is a known and specific inhibitor of phosphodiesterase type IV [the predominant isoform of phosphodiesterase found in monocytes and neutrophils]). Compounds tested are identified by the number assigned to them in Table 1. The horizontal axis of each graph shows the amount of each compound tested (in μM) while the vertical axis shows the IC_{50} values for TNF-α production as a percentage of the production in the presence of only the control compound.

FIGURE 6 is a graph depicting the results of an assay for in vivo leukopenia in mouse blood in response to lipopolysaccharide (LPS). Leukopenia induced by LPS is mediated by TNF. Hence, this model assesses both TNF production and action. Compounds tested for inhibition of leukopenia are identified by the number assigned to them in Table 1. Along the x axis of the graph, the numbers correspond to the number of white blood cells detected as cells/ml of fluid.

The results (shown by bars) are expressed in terms of a percentage of the leukopenia response (based on neutrophil content) to pure LPS, in absence of other compounds.

FIGURE 7 depicts the results of an assay for inhibition by compounds of the invention (nos. 37 and 6) of the effects of a cell permeable ceramide analog (C_2 - ceramide), dihydro ceramide and diacyl glycerol on TNF-α production by human monocytes. Inhibition of TNF-α production was measured by ELISA; the results are indicated in pg/ml of TNF-α along the x axis.

FIGURE 8 depicts the results of an assay for inhibition by a compound of the invention (no. 37) to prevent the stimulatory effects of C_2 - ceramide or protein kinase C activity in human lymphocyte extracts. Inhibitory effects were assessed as a measure of DNA synthesis; [^{3}H] thymidine incorporation detection is indicated along the y axis.

FIGURE 9 depicts the results of an assay for *in vitro* TNF-α production by human macrophages in response to lipopolysaccharide (LPS) and inhibition of that production by pteridine and isoquinolone compounds of the invention (nos. 37 and 11-49). Along the x axis of the graph, the numbers correspond to the concentration of TNF-α detected in pg/ml.

FIGURE 10 depicts inhibition of PDGF induced fibroblast proliferation among 3T3 fibroblasts in response to the inventive compounds. The compounds tested are identified along the x axis by the numbers assigned to them in Table 1. Inhibitory effects were assessed as a measure of DNA synthesis; [^{3}H] thymidine incorporation detection is indicated along the y axis.

FIGURE 11 depicts inhibition of EGF induced fibroblast proliferation among 3T3 fibroblasts in response to the inventive compounds. The compounds tested are identified along the x axis by the numbers assigned to them in Table 1. Inhibitory effects were assessed as a measure of DNA synthesis; [^{3}H] thymidine incorporation detection is indicated along the y axis.

FIGURE 12 depicts data indicative of the apoptopic protective characteristics of the compounds of the invention as represented by compounds 1C-261 (Compound 37) and 1I-49. Human lymphocytes were cultured in serum aliquots with the concentrations of the inventive compounds indicated along the x axis of the FIGURE. Protective effects were measured over 4 days as a function of the length of survival of the cultured cells in the presence of the inventive compounds as compared to survival of the cells in the absence of the inventive compounds. 100% survival (y axis) means that a number of treated cells all survived throughout the test period while an equal number of untreated cells died.

FIGURES 13(a) through **(c)** show the structure of commercially available isoquinoline structures whose inhibitory effect with respect to production of TNF-α by human monocytes prior to modification to add side chain substituents according to the invention was tested. Except for Compound S52,626-6 (6,7-dimethoxy- 1(2H)-isoquinoline, which possessed mild inhibitory activity as shown in FIGURE 1O) none of the tested compounds possessed any such inhibitory activity prior to their modification according to the invention, even at concentrations up to 500 μM.

DETAILED DESCRIPTION OF THE INVENTION

Unless otherwise noted, each of the references cited in the following disclosure is incorporated herein by reference for the purpose of illustrating the level of knowledge and skill in the art with respect to the subject matter discussed. In view of this disclosure, those of ordinary skill in the art will be familiar with, or can readily ascertain, equivalents to the techniques, compounds and other material discussed in each reference suitable for use in the invention.

I.

Composition and Activity of the Bifunctional Activation Cascade Antagonists

The bifunctional activation cascade antagonists comprise a JNK activation cascade antagonist constituent as described in commonly owned US Patent Application Serial Nos.08/858,778, 08/367,102 and 08/482,551 (which describe the constituent compounds but do not suggest their selectivity for antagonizing the JNK activation cascade) and another anti-inflammatory compound which antagonizes the p38 activation cascade. Each constituent inhibits PIC production, especially production of TNFα. Examples of JNK and p38 activation cascade antagonists are identified in Table I and compared to other anti-inflammatory agents. In the Table, the CSAID compounds which begin with "SK&F" or "SB" are manufactured by Smith-Kline

Beecham Pharmaceuticals, USA. The "FR" compound is manufactured by Fuji Laboratories, Japan. The "SR" compound is manufactured by Sanofi Recherche, France. Other compounds listed are commercially available from different sources.

It should be noted that the term "CSAID" is used in this disclosure as a generic term for p38 activation cascade antagonists, especially pyridylimidazole compounds. However, it is acknowledged that CSAID is also used as a trademark by SmithKlineBeecham Pharmaceuticals for specific pyridylimidazole compounds which antagonize p38 activation. Such compounds are included in, but do not exclusively comprise, the class of compounds referred to herein as "CSAIDs".

TABLE I

COMPOUND CLASS	ACTIVITY	EXAMPLES
JNK activation cascade antagonists	PIC inhibitors, affect JNK activation cascade	Described in US Patent Application Serial Nos.08/858,778, 08/367,102 and 08/482,551
CSAIDS	PIC inhibitors, affects p38 activation cascade	SK&F 86002 (also inhibits CO/5-LO), SK&F 104351, SK&F 105561, SK&F 105809, SK&F 202190, SB 203580, SB 206718, PD 098059 SR31747 FR133605
NSAID prosotoglandin endoperoxidase (PGHS) and 5-lipooxygenase inhibitors (combination compounds also known as cyclooxygenase(CO)/lipooxygenase (5-LO) dual inhibitors)	inhibit CO and/or 5-LO; tend to stimulate PIC production	ibuprofen naproxen
corticosteroids	PIC inhibitor	cortisone, fluticasone propionate, beclomethosone, prednisolone
phosphodiesterase (PDE) inhibitors	inhibit PDE in signalling transduction pathways	rolipram (inhibits 4-PDE) RO-1724 (same target activity)

Of the classes of compounds listed in Table I, the NSAIDs and PDE inhibitors have, respectively, undesirable PIC stimulatory activity and side-effects. Similarly, while useful, corticosteroids pose the risk of certain undesirable side-effects

and are not as specific in their PIC inhibitory activity as CSAIDs. For this reason, pyridylimidazole compounds, especially CSAIDs, are the compounds of choice for use as partners to JNK activation cascade antagonists to form the bifunctional activation cascade antagonists of the invention, which may be administered alone or together with other anti-inflammatory agents and medicaments as indicated.

Administered alone, CSAIDs inhibit 70-80% or less of TNFα release in an art-accepted model of stress-induced inflammation (Example 2). CSAIDs predominantly antagonize activation of p38 kinase, but do not affect JNK activation to a signficant degree. Conversely, the activity of the JNK activation cascade antagonist constituents of the bifunctional activation cascade antagonists of the invention is predominantly directed toward inhibition of PIC-stimulated JNK activation. These constituents of the inventive compounds have little impact on p38 activation.

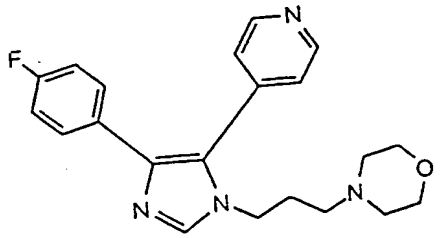
Surprisingly, combination of CSAIDs with JNK activation cascade antagonists produces a synergistic effect which gives the resulting bifunctional activation cascade antagonists greater anti-inflammatory effect than is achieved by either compound alone. For example, as measured by suppression of TNFα release in stressed cells, up to 100% inhibition is achieved by bifunctional activation cascade antagonists (Example 1).

The JNK activation cascade antagonist and CSAID constituents of the bifunctional activation cascade antagonists of the invention antagonize activation of different MAPK pathways in a non-overlapping manner. While the invention is not to be limited by any particular theory as to its mechanism of action, it is likely that the increased potency of bifunctional activation cascade antagonists as compared to their

constituent components is owing to a synergism produced by their non-overlapping effects on both of the PIC-sensitive MAPK signalling pathways. Thus, the bifunctional activation cascade antagonists of the invention exert synergistically enhanced, non-additive, pathway-specific effects on the JNK and p38 activation cascade pathways. As such, the compounds can provide greater anti-inflammatory potency, with relatively low risk of additive toxicity, than would be expected from a combination of anti-inflammatory agents.

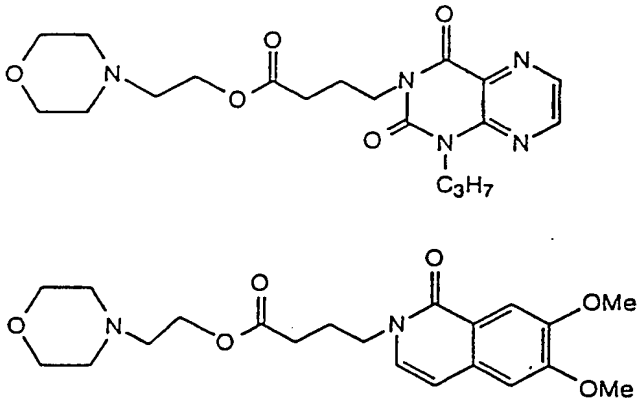
Descriptions of representative CSAIDs and JNK activation cascade antagonists useful as constituents of the bifunctional activation cascade antagonists of the invention are provided in Section II, A and B of this disclosure. For example, CSAID SB 203580 (inhibits inflammation in several animal models; Badger, *et al.*, *J.Pharmacol.Exp.Ther.*, 3:1453-1461, 1996) and SB 210313 (Boehm, *et al.*, *J.Med.Chem.*, 39:3929-3927, 1996). Particularly potent JNK activation cascade antagonist constituents are compounds **54** and **54a** (morpholinyl esters of Compound 52, described in Example XVII; isoquinilones), compound 6 (a purine) or compound 37 (a pteridine). Representative structures for each of these high potency constitutents are:

(1)



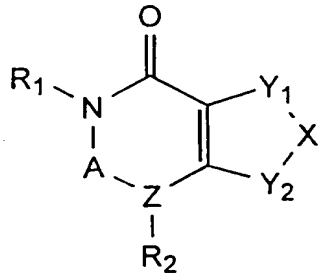
CSAID SB203580

(2)



COMPOUNDS 54 and 54a

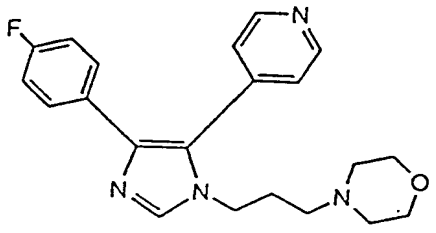
(3)



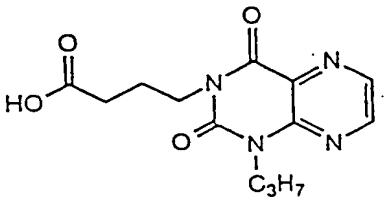
Where R_2 is $(CH_2)_3$ COOEt

COMPOUND 6

(4)



CH_3CHO



COMPOUND 37

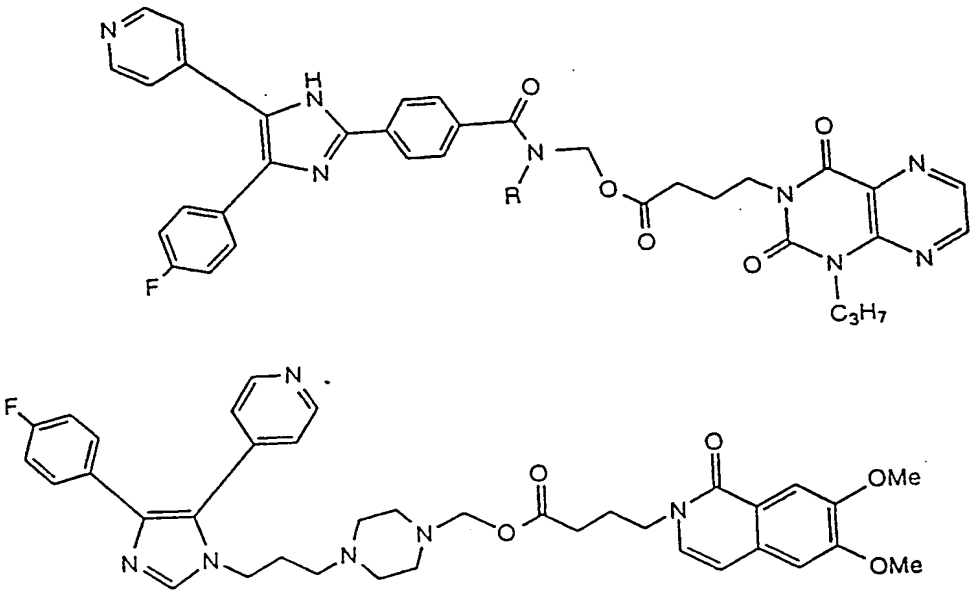
The constituents of the bifunctional activation cascade antagonists may be co-administered as separate compounds. However, to ameliorate their systemic activity, the constituents may be conjugated to one another by a bond which is severable *in vivo* and delivered as a prodrug or combined as separate constituents within a single unit delivery vehicle, such as a liposome.

Suitable conjugation means include amide, ester and aso linkages, which are separable *in vivo* by, for example, the action of proteinases or esterases. A particularly useful conjugation bond that is cleavable *in vivo* is a tertiary N acyloxymethyl amide bond (Moreira, *et al., Tetrahedron Lett.*, 35:7107-7110 (1994), the disclosure of which is incorporated herein to illustrate the steps for formation of such bonds).

Briefly, tertiary N acyloxymethyl amide bonds are formed to stabilize carboxylic acid or secondary amide moities on prodrugs (i.e., drugs bound to inert carriers by a bond which is separable *in vivo*). The bonds involve direct coupling of a $R_1CoNR_2CH_2$ (secondary amide) moiety to an R_3CO_2H drug.

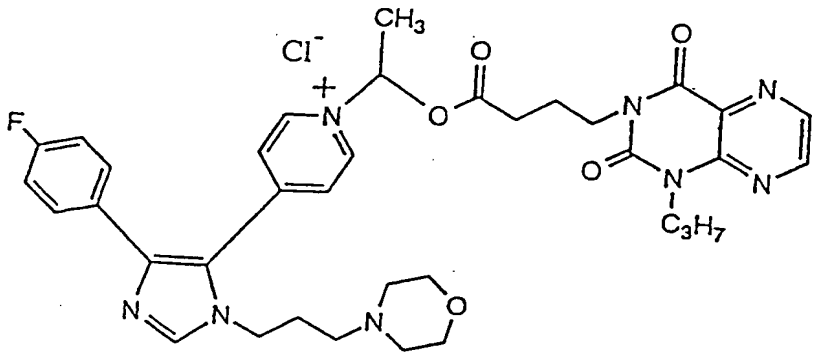
Examples of bifunctional activation cascade antagonists bound by ester-linkages are:

(5)



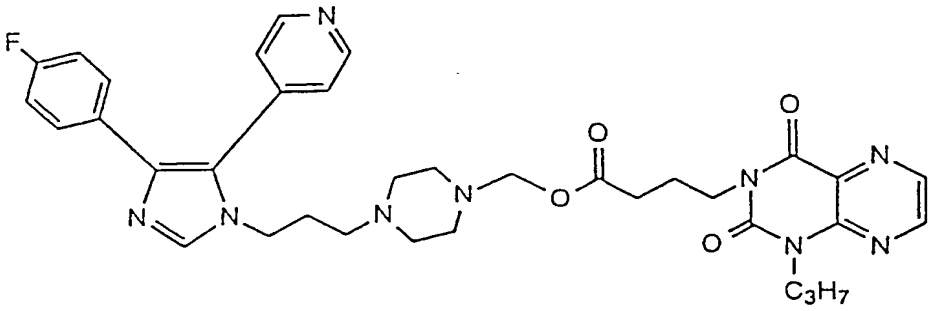
SB203313/COMPOUND 54 or 54a

(6)



SB203580/COMPOUND 37

(7)



SB203313/COMPOUND 37

wherein, for all compounds, n is any number of carbon atoms from 1 to 7, O or N;

R_1, if present, is H, an alkyl, a cyclic alkyl, a heterocyclic alkyl, alkenyl, or aralkyl having less than 7 carbon atoms;

R_6 and R_7 are H, OH or OR_1, in any combination;

Z is N or C;

X, where Z is C, is H, halogen, N_3, NO, NH_2, NHR_1, $N(R_1)2$ or COR_1; and,

A is H, halogen, N_3, NO, NH_2, NHR_1, $N(R_1)2$ or COR_1.

The bifunctional activation cascade antagonists of the invention may be prepared in a pharmaceutically acceptable carrier. Pharmaceutically acceptable carriers preferred for use with the bifunctional activation cascade antagonists of the invention may include sterile aqueous of non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/ aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's or fixed oils. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers (such as those based on Ringer's dextrose), and the like. Preservatives and other additives may also be present such as, for example, antimicrobials, antioxidants, chelating agents, and inert gases and the like. A composition of bifunctional activation cascade antagonists may also be lyophilized using means well known in the art, for subsequent reconstitution and use according to the invention.

Absorption promoters, detergents and chemical irritants (e.g., keritinolytic agents) can enhance transmission of a bifunctional activation cascade antagonist to a target tissue. For reference concerning general principles regarding absorption promoters and detergents which have been used with success in mucosal delivery of organic and peptide-based drugs, see Chien, *Novel Drug Delivery Systems*, Ch. 4 (Marcel Dekker, 1992).

Examples of suitable nasal absorption promoters in particular are set forth at Chien, *supra* at Ch. 5, Tables 2 and 3; milder agents are preferred. Suitable agents

for use in the method of this invention for mucosal/nasal delivery are also described in Chang, *et al., Nasal Drug Delivery*, "Treatise on Controlled Drug Delivery", Ch. 9 and Table 3-4B thereof, (Marcel Dekker, 1992). Suitable agents which are known to enhance absorption of drugs through skin are described in Sloan, Use of Solubility Parameters from Regular Solution Theory to Describe Partitioning-Driven Processes, Ch. 5, "Prodrugs: Topical and Ocular Drug Delivery" (Marcel Dekker, 1992), and at places elsewhere in the text. All of these references are incorporated herein for the sole purpose of illustrating the level of knowledge and skill in the art concerning drug delivery techniques.

A colloidal dispersion system may be used for targeted delivery of the bifunctional activation cascade antagonist to an inflamed tissue. Colloidal dispersion systems include macromolecule complexes, nanocapsules, microspheres, beads, and lipid-based systems including oil-in-water emulsions, micelles, mixed micelles, and liposomes. A particularly convenient and effective colloidal system is a liposome.

Liposomes are artificial membrane vesicles which are useful as delivery vehicles *in vitro* and *in vivo*. It has been shown that large unilamellar vesicles (LUV), which range in size from 0.2-4.0 μm can encapsulate a substantial percentage of an aqueous buffer containing large macromolecules. Compounds can be encapsulated within the aqueous interior and be delivered to cells in an active form. In addition to mammalian cells, liposomes have been used for delivery of polynucleotides in plant, yeast and bacterial cells.

The composition of a liposome is usually a combination of phospholipids, particularly high-phase-transition-temperature phospholipids, usually in combination

with steroids, especially cholesterol. Other phospholipids or other lipids may also be used. The physical characteristics of liposomes depend on pH, ionic strength, and the presence of divalent cations. Examples of lipids useful in liposome production include phosphatidyl compounds, such as phosphatidylglycerol, phosphatidylcholine, phosphatidylserine, phosphatidylethanolamine, sphingolipids, cerebrosides, and gangliosides. Particularly useful are diacylphosphatidylglycerols, where the lipid moiety contains from 14-18 carbon atoms, particularly from 16-18 carbon atoms, and is saturated. Illustrative phospholipids include egg phosphatidylcholine, dipalmitoylphosphatidylcholine and distearoylphosphatidylcholine.

The targeting of liposomes can be classified based on anatomical and mechanistic factors. Anatomical classification is based on the level of selectivity, for example, organ-specific, cell-specific, and organelle-specific. Mechanistic targeting can be distinguished based upon whether it is passive or active. Passive targeting utilizes the natural tendency of liposomes to distribute to cells of the reticulo-endothelial system (RES) in organs which contain sinusoidal capillaries. Active targeting, on the other hand, involves alteration of the liposome by coupling the liposome to a specific ligand such as a monoclonal antibody, sugar, glycolipid, or protein, or by changing the composition or size of the liposome in order to achieve targeting to organs and cell types other than the naturally occurring sites of localization.

The surface of the targeted delivery system may be modified in a variety of ways. In the case of a liposomal targeted delivery system, lipid groups can be

incorporated into the lipid bilayer of the liposome in order to maintain the targeting ligand in stable association with the liposomal bilayer. Various well known linking groups can be used for joining the lipid chains to the targeting ligand (see, e.g., Yanagawa, *et al., Nuc.Acids Symp.Ser.*, 19:189 (1988); Grabarek, *et al.*, *Anal.Biochem.*, 185:131 (1990); Staros, *et al., Anal.Biochem.*, 156:220 (1986) and Boujrad, *et al., Proc.Natl.Acad.Sci.USA*, 90:5728 (1993)). Examples of targeting ligands are receptor ligands, antigens, enzymes and monoclonal antibodies. Such targeting ligands can be prepared according to conventional techniques (e.g., peptide synthesis) and many are commercially available.

II.

Bifunctional Activation Cascade Antagonist Constituents

JNK Activation Cascade Antagonist Constituents

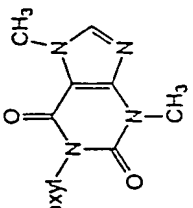
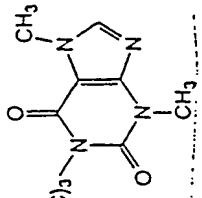
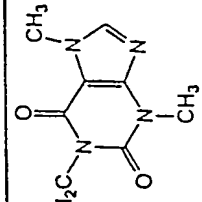
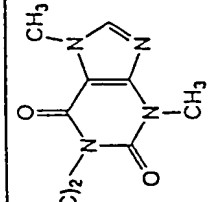
JNK activation cascade antagonist constituents useful in the invention include all of the constituents described in commonly owned US Patent Application Serial Nos.08/858,778, 08/367,102 and 08/482,551, whose disclosures are incorporated herein. For ease of reference, details of the structures of each of these compounds, as well as additional compounds of similar activity, as well as methods for their synthesis, are provided below.

JNK activation cascade antagonists of the invention generally comprise purines, pteridines, thiadiazolopyrimidines and quinazolines prepared according to the schemes described below. For reference, the techniques used in synthesizing the compounds are adaptations of the well-known Traube Synthesis protocol (Lister, *"Purines"* (Wiley-Interscience, 1971), at p. 220), beginning with 4,5-diaminopyrimidines; to wit: (1) for the purines in general, *see* Brown, *"The Chemistry of Heterocyclic Compounds: Fused Pyrimidines"*, Part II, The Purines, 1971), at pp. 31-90; (2) for the 9-dieazapurines in particular, *see* Fox, *et al., J. Org. Chem.*, 43:2536, 1978; (3) for the pteridines, *see,* for a description of the standard Timmis reaction, Nishigaki, *et al., Heterocycles,* 15:757-759, 1981; Timmis, *Nature,* 164:13 9, 1949, (or other standard Traube-like protocols for preparing pteridines by ring closure of diaminopyfimidines using a two carbon reagent); and, (4) for the pyrimidines, see, Schrage and Hitchings, *J. Org. Chem.*, 16:207, 1951.

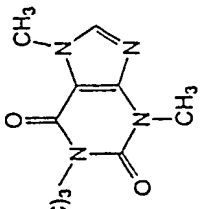
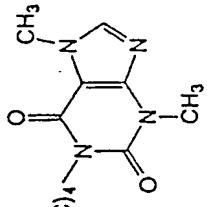
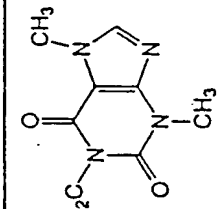
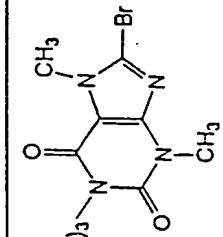
JNK activation cascade antagonists, intermediates and compounds tested for comparison of activity to the compounds of the invention are identified in the discussion below by the numbers assigned to each compound in Table 2.

TABLE 2

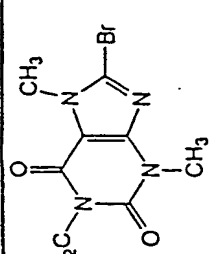
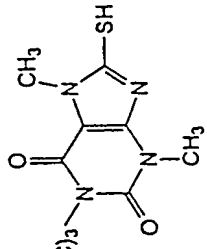
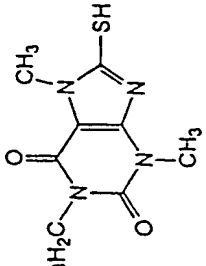
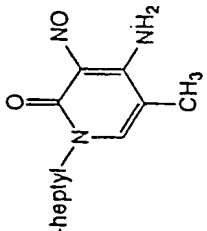
Physicochemical Data for all New Compounds and TNF-α Inhibition Data

Comp	Type		mp(°C)	formula	analyses[a]	TNFαIC50[b]
1	I	 5-oxohexyl	102-103			85
2	I	 $HOOC(H_2C)_3$	208-210			>200
4	I	 $EtOOCH_2C$	162-165	$C_{11}H_{14}N_4O_4$	C,H,N	200
5	I	 $EtOOC(H_2C)_2$	76-78	$C_{12}H_{16}N_4O_4$	HRMS	10

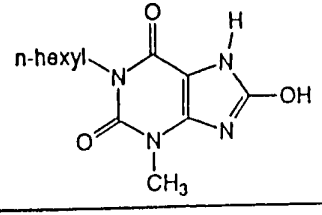
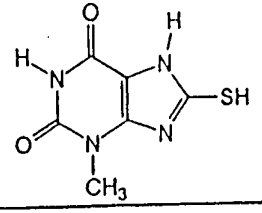
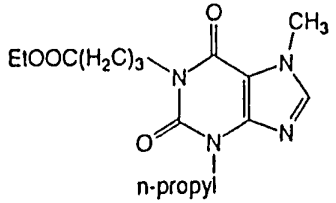
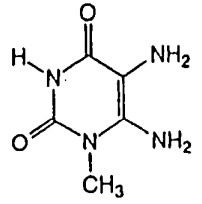
Physicochemical Data for all New Compounds and TNF-α Inhibition Data

Comp	Type		mp(°C)	formula	analyses[a]	$TNF\alpha IC_{50}$[b]
6	I		73-75	$C_{13}H_{18}N_4O_4$	C,H,N	6
7	I		84-85	$C_{14}H_{20}N_4O_4$	C,H,N	11
8	I		158-160	$C_{16}H_{16}N_4O_4$	C,H,N	>200
10	I		101-102	$C_{13}H_{17}BrN_4O_4$	C,H,N	60

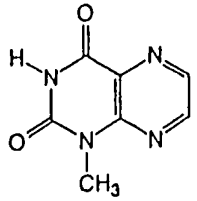
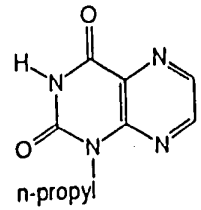
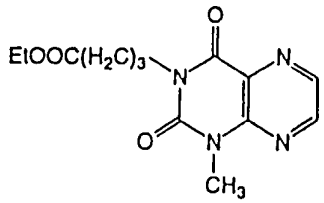
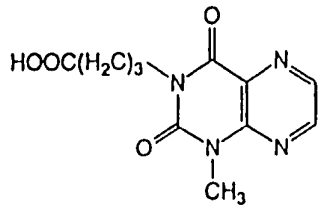
Physicochemical Data for all New Compounds and TNF-α Inhibition Data

Comp	Type		mp(°C)	formula	analyses[a]	$TNF\alpha IC_{50}$[b]
11	I		218-219	$C_{16}H_{15}BrN_4O_4$	C,H,N	>200
12	I		202-206	$C_{13}H_{18}N_4O_4S$	C,H,N	100
13	I		>293 dec	$C_{17}H_{18}N_4O_4S$	C,H,N	>200
20	V		>218 dec	$C_{12}H_{20}N_4O_3$	C,H,N	ND[c]

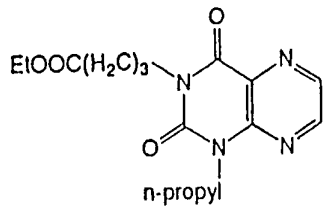
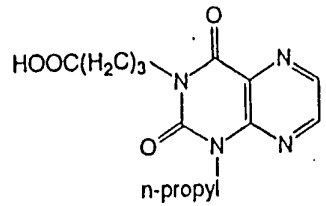
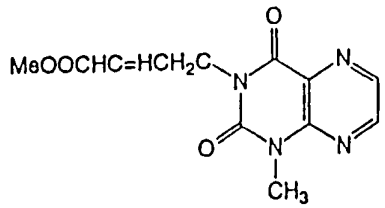
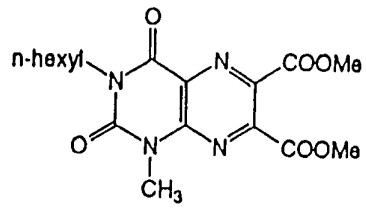
Physicochemical Data for all New Compounds and TNF-α Inhibition Data

Comp	Type		mp(°C)	formula	analyses[a]	TNFαIC$_{50}$[b]
24	I		>295 dec	$C_{12}H_{18}N_4O_3$	C,H,N	50
25	I		>320			>200
31	I		oil	$C_{15}H_{22}N_4O_4$	C,H,N	5
33	V		>270 dec	$C_5H_8N_4O_2$	C,H,N	ND

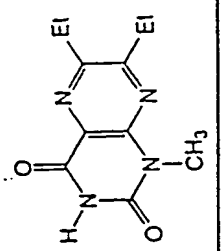
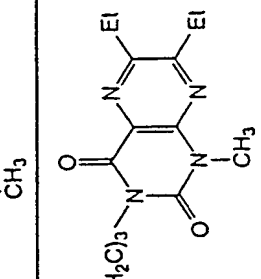
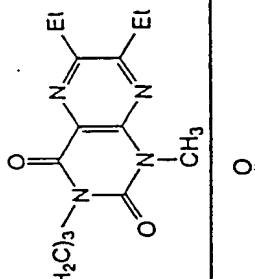
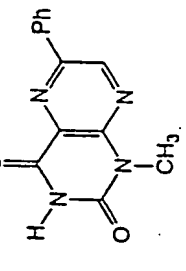
Physicochemical Data for all New Compounds and TNF-α Inhibition Data

Comp	Type		mp(°C)	formula	analyses[a]	TNFαIC$_{50}$[b]
34	II		287-289	$C_7H_6N_4O_2$	C,H,N	ND
35	II		187-189	$C_9H_{10}N_4O_4$	C,H,N	ND
36	II		90-92	$C_{13}H_{16}N_4O_4$	C,H,N	12
36a	II		199-202	$C_{11}H_{12}N_4O_4$	C,H,N	>200

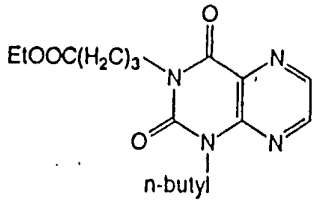
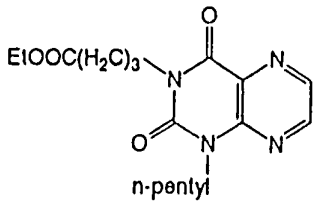
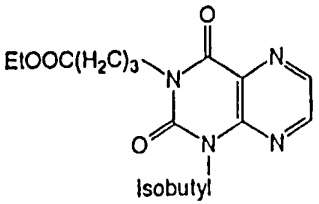
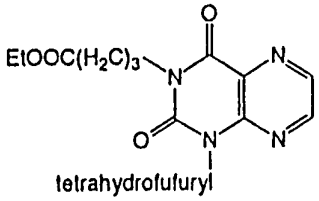
Physicochemical Data for all New Compounds and TNF-α Inhibition Data

Comp	Type		mp(°C)	formula	analyses[a]	TNFαIC$_{50}$[b]
37	II		53-55	$C_{15}H_{20}N_4O_4$	C,H,N	5
37a	II		97-99	$C_{13}H_{16}N_4O_4$	C,H,N	>200
38	II		118-119	$C_{12}H_{12}N_4O_4$	HRMS	25
39	II		92-95	$C_{17}H_{22}N_4O_6$	C,H,N	ND

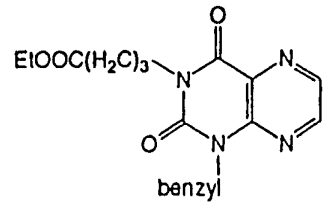
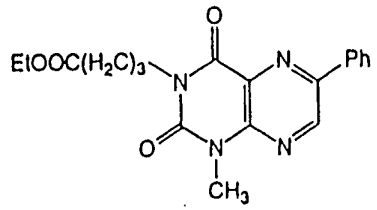
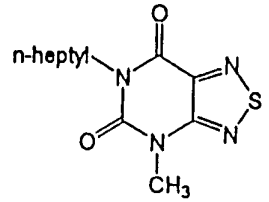
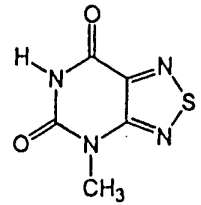
Physicochemical Data for all New Compounds and TNF-α Inhibition Data

Comp	Type		mp(°C)	formula	analyses[a]	$TNF\alpha IC_{50}$[b]
40	II		218-222	$C_{11}H_{14}N_4O_2$	C,H,N	ND
41	II		66-68	$C_{17}H_{24}N_4O_4$	C,H,N	130
41a	II		161-162	$C_{15}H_{20}N_4O_4$	C,H,N	ND
42	II		>307 dec	$C_{13}H_{10}N_4O_4$	N/A	ND

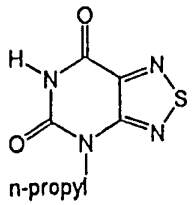
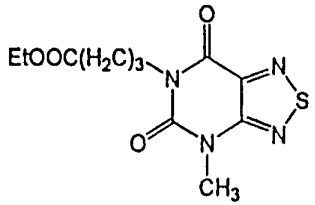
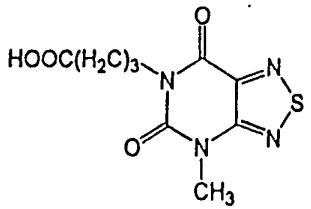
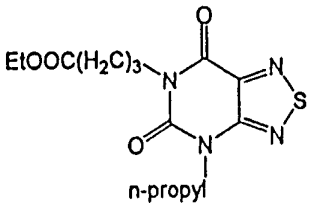
Physicochemical Data for all New Compounds and TNF-α Inhibition Data

Comp	Type		mp(°C)	formula	analyses[a]	TNFαIC$_{50}$[b]
43a	II		N/A	N/A	N/A	N/A
43b	II		N/A	N/A	N/A	N/A
43c	II		N/A	N/A	N/A	N/A
43d	II		N/A	N/A	N/A	N/A

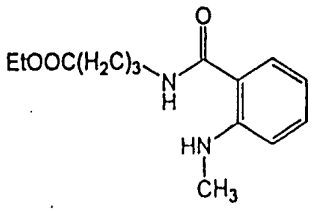
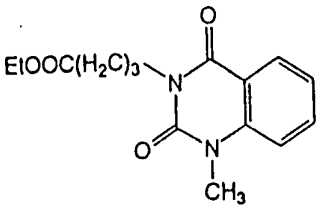
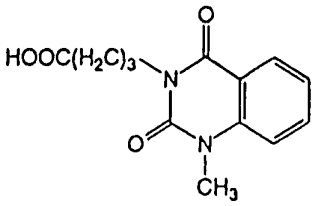
Physicochemical Data for all New Compounds and TNF-α Inhibition Data

Comp	Type		mp(°C)	formula	analyses[a]	$TNF\alpha IC_{50}$[b]
43e	II		N/A	N/A	HRMS	N/A
43	II		99-100	$C_{19}H_{20}N_4O_4$	HRMS	>200
44	III		73-75	$C_{12}H_{18}N_4O_2S$	C,H,N	75
45	III		210-213	$C_5H_4N_4O_2S$	C,H,N	ND

Physicochemical Data for all New Compounds and TNF-α Inhibition Data

Comp	Type		mp(°C)	formula	analyses[a]	TNFαIC$_{50}$[b]
46	III		142-144	$C_7H_8N_4O_2S$	C,H,N	ND
47	III		45-47	$C_{11}H_{14}N_4O_4S$	C,H,N	25
47a	III		121-122	$C_9H_{10}N_4O_4S$	HRMS	>200
48	III		oil	$C_{13}H_{18}N_2O_3$	C,H,N	18

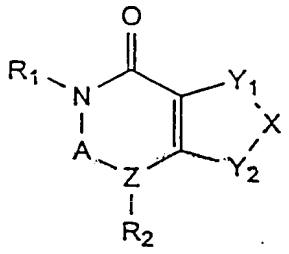
Physicochemical Data for all New Compounds and TNF-α Inhibition Data

Comp	Type		mp(°C)	formula	analyses[a]	TNFαIC$_{50}$[b]
51	IV		oil	$C_{14}H_{20}N_4O_3$	C,H,N[f]	ND
52	IV		oil	$C_{15}H_{18}N_2O_4$	C,H,N[g]	55
52a	IV		163-165	$C_{13}H_{14}N_2O_4$	C,H,N	>200

[a]All compounds analyzed for C, H, N or by exact mass high resolution mass spectrometry; results were within ±0.4% of theoretical values.[b]Concentration of compound in μM which inhibited the product of TNFα by 50% of control.[c] ND = not determined.[d] C: calc'd, 58.61; found 59.22. [e]H: calc'd, 4.13; found 3.70. [f]N: calc'd, 10.25; found 9.77. [g]C: calc'd, 59.62; found, 60.61. Compound types are: I=purines; II=pteridines; III=thiadiazolopyrimidines; IV=quinazolines; and, V=isoquinolones. "N/A" means data not available (but can be obtained through conventional analysis techniques).

Purines

Purines JNK activation cascade antagonists have the general formula (8):



where Z is N or CH and R_1 is $(CH_2)_nA$, where:

A is NH_2, acyloxy, SO_3H, PO_4H_2, NNO(OH), SO_2NH_2, $PO(OH)NH_2$, SO_2R, or COOR where R is H, an alkyl having from 1 to 4 carbon atoms, an alkenyl having from 1 to 4 carbon atoms, tetrazolyl or benzyl;

n is any number of atoms from 1 to 7 having saturated and/or unsaturated carbon to carbon bonds, which atoms may include an oxygen or nitrogen atom in place of a carbon atom to form, respectively, ether or amino linkages;

and, preferably, R_1 is a ω-carboxyalkyl, ω-carboxyalkenyl, or ω-carboxyaryl having from 1 to 8 carbon atoms, wherein the aromatic group further has as a substituent A (as defined above);

R_2 is H, an alkyl (including aliphatic and alicyclic, and heteroalicyclic forms), alkenyl, aralkyl having 1 to 7 carbon atoms or a ω-hydroxyalkyl having from 1 to 7 carbon atoms;

R_3 is the same as R_2; and

X is H, any halogen, OH, SH, OR', or SR', where R' is an alkyl, alkenyl, phenyl or benzyl having from 1 to 4 carbon atoms.

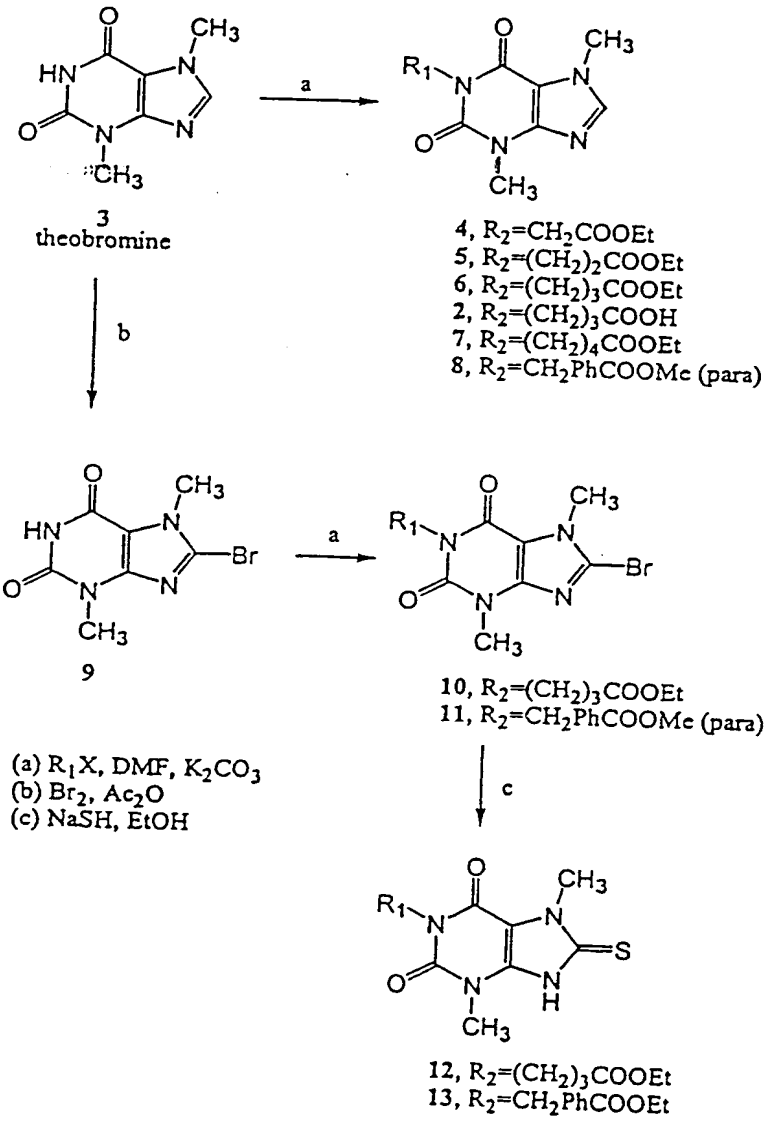
These compounds are synthesized per the below synthesis scheme (as described in further detail in the Examples).

SCHEME 1

(PURINES)

Three basic synthesis protocols were utilized in Scheme I; to wit:

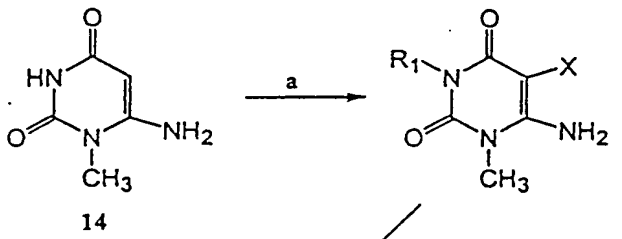
Method A



Generally, theobromine was used as the starting material under conditions to ensure N-1 alkylation took place in lieu of O-6 alkylation. Compounds 4 through

8, 10 and 11 (Table I) were prepared by this method. Compounds 10 and 11 in particular were prepared by a variation of the alkylation method in which theobromine was first brominated to give 8-bromotheobromine (compound 9), then alkylated. The 8-bromo substituent was also displaced by NaSH to yield the corresponding 8-thioxo derivatives, compounds 12 and 13.

Method B



14

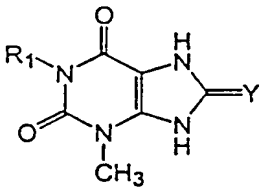
	X	R_1
15,	H	n-propyl
16,	H	n-hexyl
17,	H	n-heptyl
18,	N=O	n-propyl
19,	N=O	n-hexyl
20,	N=O	n-heptyl

b, c

22

21,	NH_2	n-propyl
22,	NH_2	n-hexyl
23,	NH_2	n-heptyl

d or a



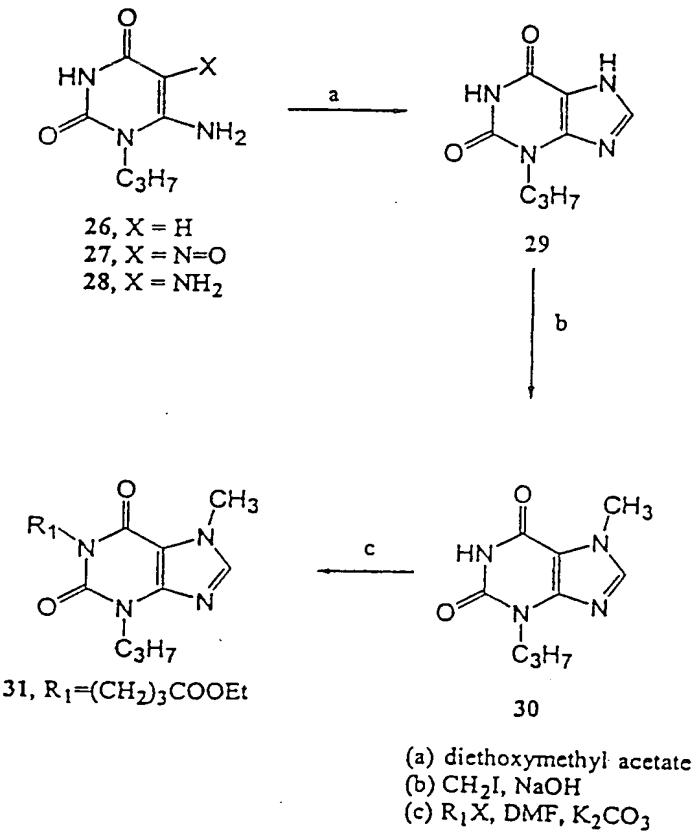
24, R_1=n-hexyl Y=O
25, R_1=H Y=S

(a) R_1X, DMF, K_2CO_3
(b) $NaNO_2$, HCl
(c) Pd/H_2
(d) urea, 140C
(e) potassium ethyl xanthate

This method is essentially based on the Traube purine synthesis protocol referred to *supra*. The method was used to prepare 1,3,8-trisubstituted xanthines bearing no alkyl group at the N-7 position. In this procedure, the N-1 substituted pyrimidine was alkylated at position N-3. Formation of the purine ring was complete

by nitrosation, reduction of the nitroso to the amine by catalytic hydrogenation, then ring closure using urea or potassium ethyl xanthate to provide compounds 24 and 25 (respectively, 8-oxo and 8-thioxo derivatives). A detailed description of this protocol is provided in the Examples.

Method C

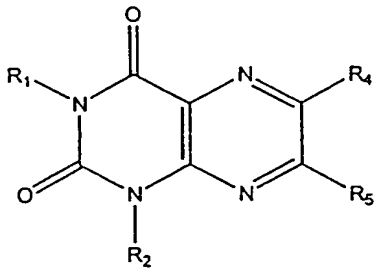


The above method was utilized to prepare the N-3 propylpurines. The starting material used was n-propyl urea condensed with ethyl cyanoacetate in the presence of sodium ethoxide to yield the 6-amino-1-propylpyrimidinedione in moderate yield. Commercially available 3-n-propylxanthine could also be used as the starting material.

Ring closure was accomplished as described in method A, except that diethoxymethyl acetate was used as the source of carbon in the ring closure step. Sequential alkylations were then performed using alkyl halides to yield the final compound 31 (ethyl 4-(2,3,6,7-tetrahydro-2,6-dioxo-7-methyl-3-n-propyl-1H-purin-1-yl)butonoic acid). A detailed description of this protocol is provided in the Examples.

Pteridines

Pteridine JNK activation cascade antagonists have the general formula (9):



R_1 is $(CH_2)_nA$, where:

A is NH_2, acyloxy, SO_3H, PO_4H_2, NNO(OH), SO_2NH_2, $PO(OH)NH_2$, SO_2R, or COOR where R is H, an alkyl having from 1 to 4 carbon atoms, an alkenyl having from 1 to 4 carbon atoms, tetrazolyl or benzyl;

n is any number of atoms from 1 to 7 having saturated and/or unsaturated carbon to carbon bonds, which atoms may include an oxygen or nitrogen atom in place of a carbon atom to form, respectively, ether or amino linkages;

and, preferably, R_1 is a ω-carboxyalkyl, ω-carboxyalkenyl, or ω-carboxyaryl having from 1 to 8 carbon atoms, wherein the aromatic group further has as a substituent A (as defined above);

R_2 is H, an alkyl (including aliphatic and alicyclic, and heteroalicyclic forms), alkenyl, aralkyl having 1 to 7 carbon atoms or a ω-hydroxyalkyl having from 1 to 7 carbon atoms;

R_4 is the same as R_2, OH or an O-alkyl having from 1 to 5 carbon atoms;

R_5 is the same as R_2, OH or an O-alkyl having from 1 to 5 carbon atoms;

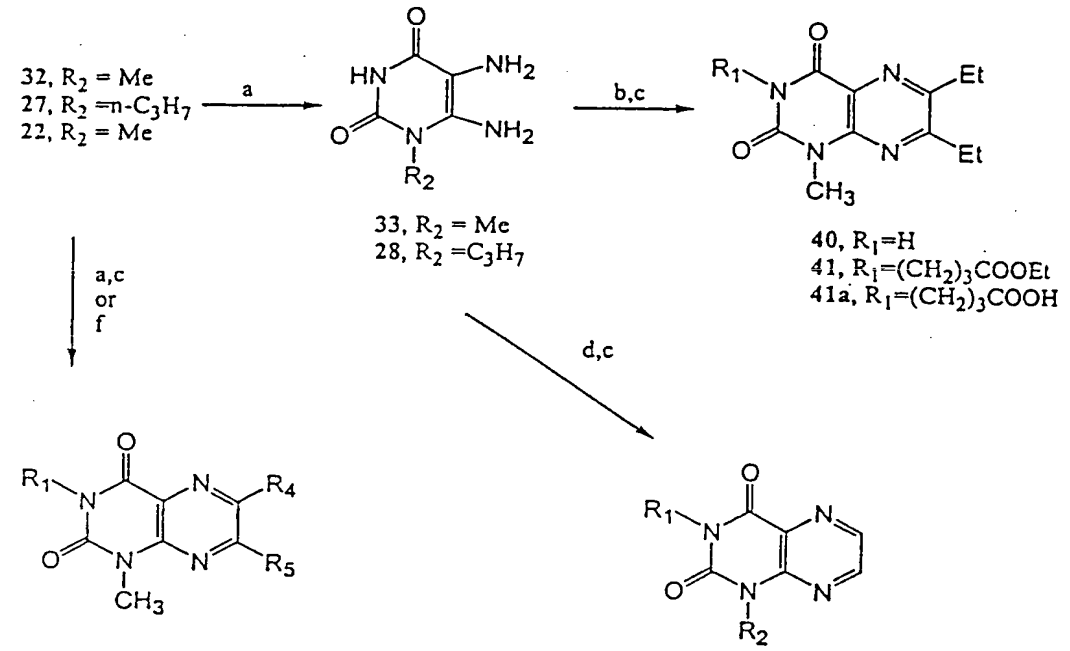
and,

Z is N or CH.

These compounds are synthesized per the below synthesis scheme (which is described in further detail in the Examples.)

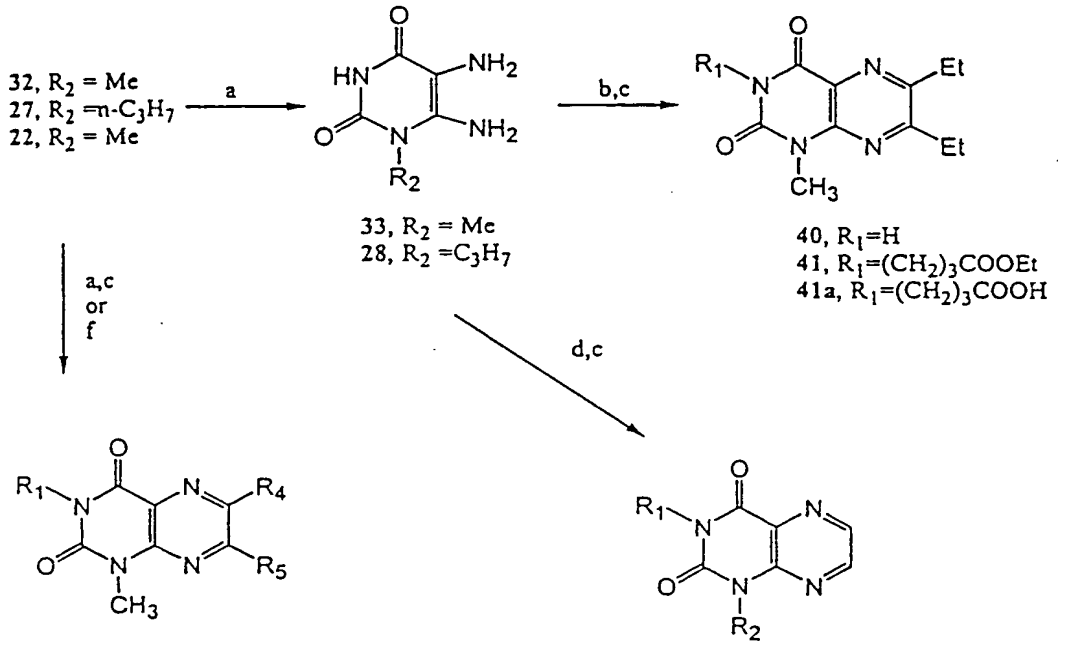
SCHEME II

(PTERIDINES)



(a) $Na_2S_2O_4$, H_2O
(b) 3,4-hexandione
(c) R_1X, DMF, K_2CO_3
(d) glyoxal:sodium bisulfite
(e) phenethylamine
(f) dimethylacetylene dicarboxylate

Method C (above) was chosen as a convenient method to produce N-alkyls in preference to O-alkyls in this group. Method C was modified to this end as follows:

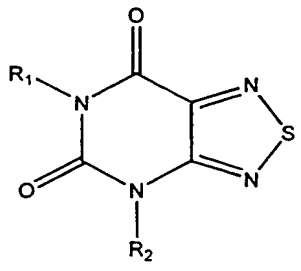


(a) $Na_2S_2O_4$, H_2O
(b) 3,4-hexandione
(c) R_1X, DMF, K_2CO_3
(d) glyoxal:sodium bisulfite
(e) phenethylamine
(f) dimethylacetylene dicarboxylate

Synthesis of the pteridines was based on orthodiaminopyrimidines as precursors. Ring closure of the orthodiamines (compounds 33 and 28) was accomplished with a two carbon source (e.g., glyoxal) to produce compounds 34 and 35 (N-1 substituted pteridines). Alkylation at N-3 as described with respect to Method A produced the desired pteridines (compounds 36-38). Further, use of 3,4-hexanedione in the ring closure step produced a more lipophilic derivative (compound 41; 6,7-diethyl pteridine). Condensation of compound 22 with dimethylacetylene dicarboxylate formed compound 39 (1,3-dialkylpteridine), while treatment of compound 27 phenethyl amine followed by alkylation provided compound 43 (6-phenyl dialkyl pteridine). Both of the latter protocols utilized a Timmis reaction to produce the desired products. A detailed description of these protocols is provided in the Examples.

Thiadiazolopyrimidines

Thiadiazolopyrimidine JNK activation cascade antagonists have the general formula (10):



R_1 is $(CH_2)_nA$, where:

A is NH_2, acyloxy, SO_3H, PO_4H_2, NNO(OH), SO_2NH_2, $PO(OH)NH_2$, SO_2R, or COOR where R is H, an alkyl having from 1 to 4 carbon atoms, an alkenyl having from 1 to 4 carbon atoms, tetrazolyl or benzyl;

n is any number of atoms from 1 to 7 having saturated and/or unsaturated carbon to carbon bonds, which atoms may include an oxygen or nitrogen atom in place of a carbon atom to form, respectively, ether or amino linkages; and

and, preferably, R_1 is a ω-carboxyalkyl, ω-carboxyalkenyl, or ω-carboxyaryl having from 1 to 8 carbon atoms, wherein the aromatic group further has as a substituent A (as defined above); and

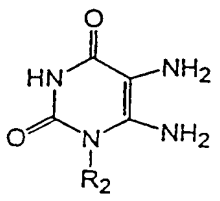
R_2 is H, an alkyl (including aliphatic and alicyclic, and heteroalicyclic forms), alkenyl, aralkyl having 1 to 7 carbon atoms or a ω-hydroxyalkyl having from 1 to 7 carbon atoms.

These compounds are synthesized per the below synthesis scheme (as described in further detail in the Examples).

SCHEME III

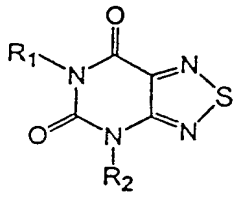
(THIADIAZOLOPYRIMIDINES)

Method C (above) was chosen as a convenient method to produce N-alkyls in preference to O-alkyls in this group. Method C was modified to this end as follows:



33, R_2 = Me
28, R_2 = C_3H_7

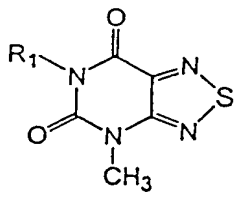
a,b



45, R_1=H R_2=Me
46, R_1=H R_2=C_3H_7
47, R_1=$(CH_2)_3COOEt$ R_2=Me
47a, R_1=$(CH_2)_3COOH$ R_2=Me
48, R_1=$(CH_2)_3COOEt$ R_2=C_3H_7

23

a,b

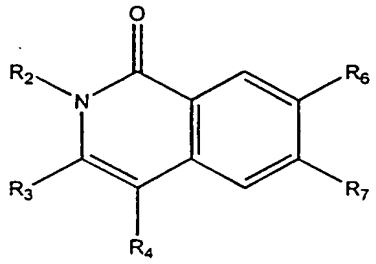


44, R_1=n-hexyl

Synthesis of the pyrimidines was based on orthodiaminopyrimidines as precursors. Ring closure of the orthodiamines was accomplished by treatment with thionyl chloride in the presence of pyridine. Alkylation of these intermediates produced compounds 47 and 48 (disubstituted pyrimidines). A detailed description of this protocol is provided in the Examples.

Isoquinolones

Isoquinolone JNK activation cascade antagonists have the general formula (11):



R_2 is $(CH_2)_nA$, where:

A is NH_2, acyloxy, SO_3H, PO_4H_2, NNO(OH), SO_2NH_2, $PO(OH)NH_2$, SO_2R, or COOR where R is H, an alkyl having from 1 to 4 carbon atoms, an alkenyl having from 1 to 4 carbon atoms, tetrazolyl or benzyl;

n is any number of atoms from 1 to 7 having saturated and/or unsaturated carbon to carbon bonds, which atoms may include an oxygen or nitrogen atom in place of a carbon atom to form, respectively, ether or amino linkages;

and, preferably, R_2 is a ω-carboxyalkyl, ω-carboxyalkenyl, or ω-carboxyaryl having from 1 to 8 carbon atoms, wherein the aromatic group further has as a substituent A (as defined above); and

R_3 is H, an alkyl (including aliphatic and alicyclic, and heteroalicyclic forms), alkenyl, aralkyl having 1 to 7 carbon atoms or a ω-hydroxyalkyl having from 1 to 7 carbon atoms;

R_4 is H, OH, NH_2 or O-alkyl having from 1-7 carbon atoms;

R_5 is H, OH, NO, NO_2, NH_2 an O-alkyl having from 1-4 carbon atoms, or X where;

X is H, any halogen, OH, SH, OR', or SR', where R' is an alkyl, alkenyl, phenyl or benzyl having from 1 to 4 carbon atoms; and,

R_7 is H, OH, NO, NO_2, NH_2 an O-alkyl having from 1-7 carbon atoms, or X where:

X is H, any halogen, OH, SH, OR', or SR', where R' is an alkyl, alkenyl, phenyl or benzyl having from 1 to 7 carbon atoms.

These compounds were synthesized by purchasing isoquinolines from Aldrich Chemical and adding side chains to the ring structure with respect to the compounds of the invention. Only the 6,7-dimethoxy-1(2H)-isoquinoline compound

(Aldrich # S52,626-6) had any inhibitory effect on TNF-α production prior to addition of the side chains described above.

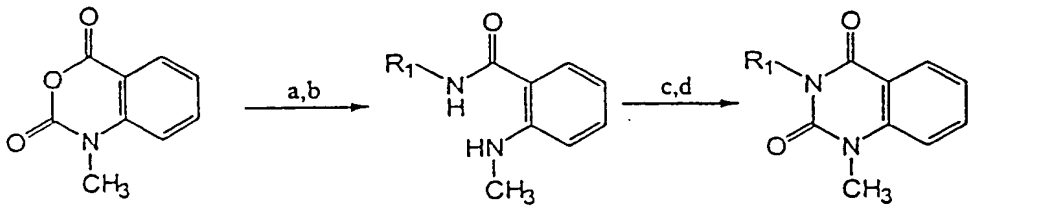
Quinazolines

Quinazoline JNK activation cascade antagonists are synthesized according to the following scheme and are represented in structure by compound **52**:

Scheme IV

(DIDEAZAPTERIDINES OR QUINAZOLINES)

Compound **52** (a quinazoline derivative; ethyl 4-(1-methyl-2,4-dioxoquinazol-3-yl)butanoic acid) was produced as follows:



49

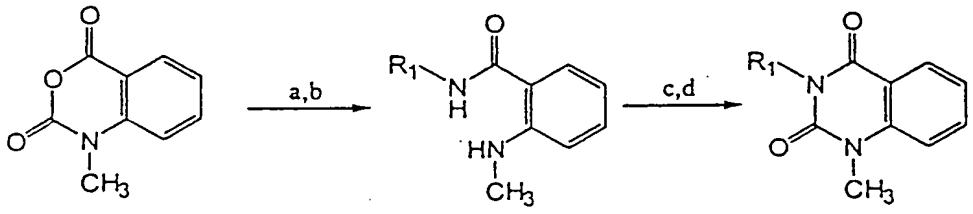
50, $R_1=(CH_2)_3COOH$
51, $R_1=(CH_2)_3COOEt$

52, $R_1=(CH_2)_3COOEt$
52a, $R_1=(CH_2)_3COOH$

The starting material for this protocol was N-methyl isatoic anhydride. A detailed description of this protocol is provided in the examples.

Increasing water solubility

The carboxylic ester and acid derivatives of the compounds described above can be converted to substituted carboxylic esters, where the substituent of the ester increases the water solubility of the compounds.



49

50, $R_1=(CH_2)_3COOH$
51, $R_1=(CH_2)_3COOEt$

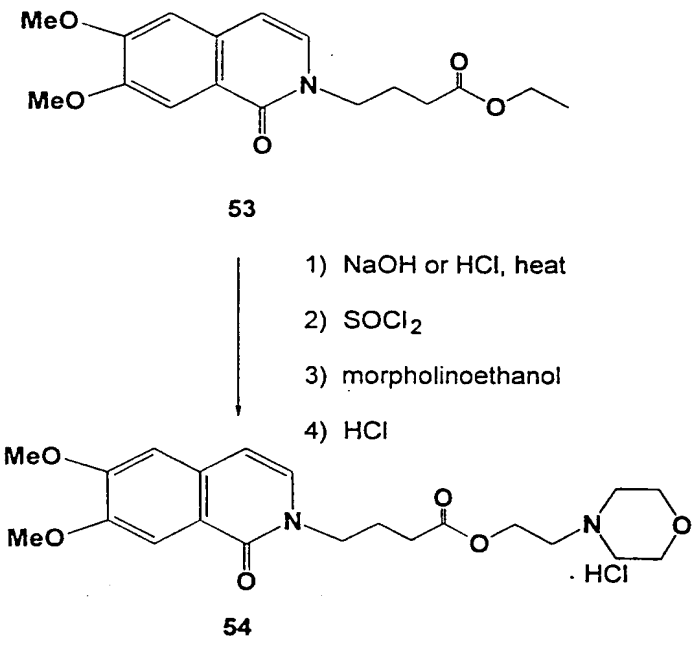
52, $R_1=(CH_2)_3COOEt$
52a, $R_1=(CH_2)_3COOH$

Particular substituents that increase the water solubility of the compounds effectively are aminoalkyl esters. The amino group of the aminoalkyl ester preferably is a secondary or tertiary amino group. The amino substituents have between 1 and 6 carbon atoms, one of which can be replaced by an oxygen atom or nitrogen atom. The preferred aminoalkyl is a tertiary amine and the amino substituents are preferably are ether groups. The most preferred aminoalkyl is N-morpholinoethyl. The carboxylic ester and carboxylic acid derivatives can be converted to the morpholinoethyl ester form by the method shown in Scheme V.

Compound 53 (an isoquinolone derivative; ethyl (6,7-dimethoxy-1(2*H*)-isoquinolonyl)butanoic acid was converted to compound 54, a morpholinoethyl ester (isolated as a hydrochloride salt) as follows:

Scheme V

(MORPHOLINOETHYL DERIVATIVES: COMPOUND 54)



CSAID Constituents

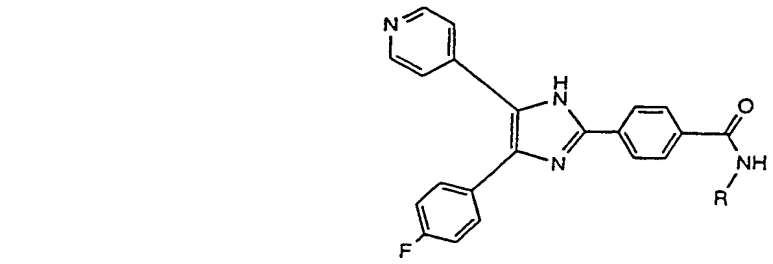
CSAIDs are available from, for example, the sources listed in the legend to Table 1. Chemical descriptions and data demonstrating activity for many of these compounds have been published in the art in, for example, US Patent No. 5,658,903; US Patent No. 5,593,992; US Patent No. 5,317,019; US Patent No. 4,794,114; US Patent No. 4,778,806; Published PCT Application No. WO9725045; Published PCT Application WO9725047; Published PCT Application WO9725048; Published PCT Application No. WO9640143; European Patent No. 565582; Austrian Patent No. 586907; Griswold, *et al., Drugs Exp.Clin.Res.*, 19:243-248 (1993)(SK&F 86002, 105809 and 104351; *in vivo*=32, 48 and 34 mg/kg, respectively); Marshall, *et al., Biochem.Pharmacol.*, 42:813-824 (1991) (SK&F 105809; *in vitro* data for inhibition of CO, 5-LO, IL-1 and PGHS); Griswold, *et al., J.Immunol.Methods*, 195:1-5 (1996) (SB 203580); and Badger,*et al.,J.Pharmacol.Exp.Ther.*, 279:1453-1461 (1996) (*in vivo* data for SB 203580); Boehm, *et al., J.Med.Chem.*, 39:3929-3937 (1997) (SB 210313)). Each of these references are incorporated herein for ease of reference in identifying CSAIDs for use in the invention.

For further ease of reference, the chemical names of several known CSAIDs are set forth below:

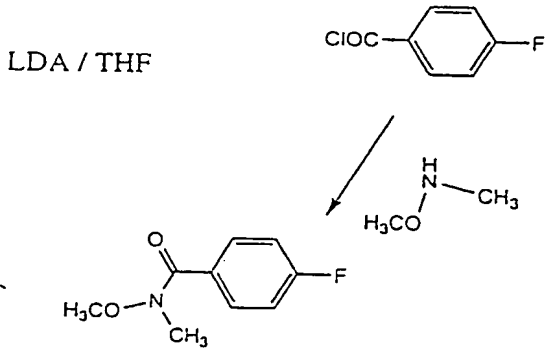
SB 210313: 1-[3-(4-morpholinyl)propyl]-4-(4-fluorophenyl-5-(4-pyridyl)imidazole

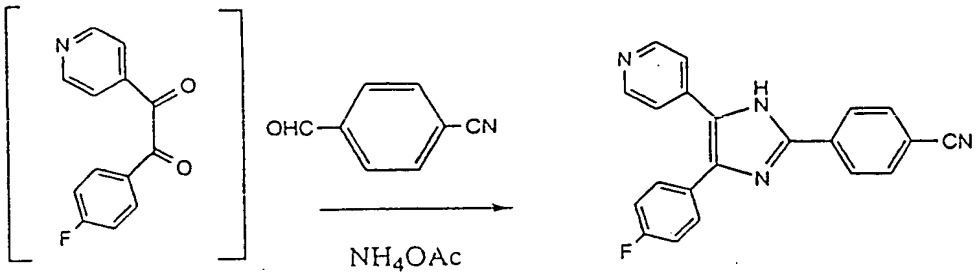
SK&F 105809:	2-(4-methylsulfinylphenyl)-3-(4-pyridyl)-6,7-dihydro-[5H]-pyrrolo[1,2-a]imidazole
SK&F 105561:	2-(4-methylthiophenyl)-3-(4-pyridyl)-6,7-dihydro-[5H]-pyrrolo[1,2-a]imidazole
SB 203580:	4-(4-fluorophenyl)-2-(4-methylsulfinyl)-5-(4-pyridyl)imidazole
SK&F 86002:	5-(4-pyridyl)-6(4-flurophenyl)-2,3-dihydroimidazole(2,1-b)thia zol

A representative CSAID synthesis scheme (shown for SB 210313) is:

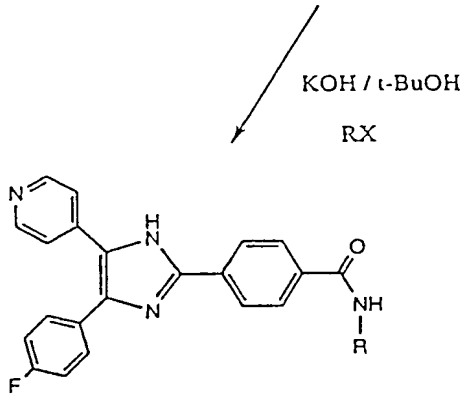


4-pyridylcarbinol





T.F. Gallagher, et al. *Bioorg. Med. Chem. Lett.* 5, 1171-1176, 1995 and US Patent 5656644.



Although not preferred as partners for JNK activation cascade antagonists in the bifunctional activation cascade antagonists of the invention, corticosteroids and other medicaments, such as antibiotics or different anti-inflammatory agents, may also be administered with the inventive compounds if clinically indicated.

III.

Kits for Use in Practicing the Methods of the Invention

For use in the methods described below, kits are also provided by the invention. Such kits may include any or all of the following: a bifunctional activation cascade antagonist; a pharmaceutically acceptable carrier (may be pre-mixed with the bifunctional activation cascade antagonist) or suspension base for reconstituting lyophilized bifunctional activation cascade antagonists; additional medicaments; a sterile vial for each bifunctional activation cascade antagonist and additional medicament (if any), or a single vial for mixtures thereof; device(s) for use in delivering the composition to a host; assay reagents for detecting indicia that the anti-inflammatory sought have been achieved in treated animals and a suitable assay device.

IV.

Methods for Use of the Inventive Compounds.

The bifunctional activation cascade antagonists of the invention may be administered to a mammalian host to retard cellular responses associated with TNF-α and IL-1 production, release and MPAK system activation. As exemplified herein, the methods of the invention are expected to be of particular use in providing

protection against inflammation and associated excess formation of fibrotic tissue. In particular, with reference to demonstrated efficacy for the JNK activation cascade antagonist and CSAID constituents of bifunctional activation cascade antagonists, these compounds can be expected to have therapeutic efficacy in the treatment of arthritis, spondylitis, bone resorption (osteoarthritis), sepsis, septic shock, endotoxic shock, inflammatory bowel disease, asthma, bronchitis, chronic pulmonary inflammatory disease, silicosis, repurfusion injury, graft versus host reachtions, allograft rejection, fever, viral and bacterial infection, inflammation of the joints, psoriasis and eczema, radiation dermatitis, cell senescence and apoptosis, multiple sclerosis and other inflammatory conditions.

The bifunctional activation cascade antagonists of the invention are administered to a host using any available method and route suitable for drug delivery, including *ex vivo* methods, as well as systemic or localized routes. Those of ordinary skill in the clinical arts will be familiar with, or can readily ascertain, means for drug delivery, which are briefly discussed below.

The entrance point for many inflammatory stimulants into a host is through the skin or mucosa. Thus, delivery methods and routes which target the skin (e.g., for cutaneous and subcutaneous conditions) or mucosa (e.g., for respiratory, ocular, lingual or genital conditions) will be especially useful.

Intranasal administration means are particularly useful in addressing respiratory inflammation, particularly inflammation mediated by antigens transmitted

from the nasal passages into the trachea or broncheoli. Such means include inhalation of aerosol suspensions or insufflation. Nebulizer devices suitable for delivery of drug compositions to the nasal mucosa, trachea and bronchioli are well-known in the art and will therefore not be described in detail here. For general review in regard to intranasal drug delivery, those of ordinary skill in the art may wish to consult Chien, *Novel Drug Delivery Systems*, Ch. 5 (Marcel Dekker, 1992).

Dermal routes of administration, as well as subcutaneous injections, are useful in addressing allergic reactions and inflammation in the skin. Examples of means for delivering drugs to the skin are topical application of a suitable pharmaceutical preparation, transdermal transmission, injection and epidermal administration.

For transdermal transmission, absorption promoters or iontophoresis are suitable methods. For review regarding such methods, those of ordinary skill in the art may wish to consult Chien, *supra* at Ch. 7. Iontophoretic transmission may be accomplished using commercially available "patches" which deliver their product continuously via electric pulses through unbroken skin for periods of several days or more. Use of this method allows for controlled transmission of pharmaceutical compositions in relatively great concentrations, permits infusion of combination drugs and allows for contemporaneous use of an absorption promoter.

An exemplary patch product for use in this method is the LECTRO PATCH trademarked product of General Medical Company of Los Angeles, CA. This product electronically maintains reservoir electrodes at neutral pH and can be adapted to provide dosages of differing concentrations, to dose continuously and/or to dose periodically. Preparation and use of the patch should be performed according to

the manufacturer's printed instructions which accompany the LECTRO PATCH product; those instructions are incorporated herein by this reference.

Opthalmic administration (e.g., for treatment of allergic conjunctivitis) involves invasive or topical application of a pharmaceutical preparation to the eye. Eye drops, topical cremes and injectable liquids are all examples of suitable mileaus for delivering drugs to the eye.

Systemic administration involves invasive or systemically absorbed topical administration of pharamaceutical preparations. Topical applications as well as intravenous and intramuscular injections are examples of common means for systemic administration of drugs.

The compounds of the invention vary in potency. A summary of the potency of each JNK activation cascade antagonist (expressed as a percentage of inhibition of intracellular responses to LPS, namely, the production of TNF-α, where responses to pure LPS=100% and are measured as the concentration of the inventive compound needed to inhibit TNF-α production by 50%) is provided in Table 2, elsewhere above.

The relative potencies of other antiinflammatory constituents, including CSAIDs, are known in the art. For example, the IC_{50} (level at which 50% of TNFα release is inhibited) for CSAIDs are published (see, e.g., Griswold, *et al., Drugs Exp.Clin.Res.*, 19:243-248 (1993)(SK&F 86002, 105809 and 104351; *in vivo*=32, 48 and 34 mg/kg, respectively); Marshall, *et al., Biochem.Pharmacol.*, 42:813-824 (1991) (SK&F 105809; *in vitro* data for inhibition of CO, 5-LO, IL-1 and PGHS); Griswold, *et al., J.Immunol.Methods*, 195:1-5 (1996) (SB 203580); and Badger, *et al.,*

J.Pharmacol.Exp.Ther., 279:1453-1461 (1996) (*in vivo* data for SB 203580); Boehm, *et al.*, *J.Med.Chem.*, 39:3929-3937 (1997) (SB 210313)).

The maximum potency of any of these compounds administered alone is about 70-80% TNFα inhibition (Example I). Those of ordinary skill in the art will recognize that lesser or greater dosages of the compounds of the invention may be required depending on the potency of the particular compound being administered.

Dosages of the compounds of the invention will vary depending on the age, weight and presenting condition of the host to be treated, as well as the potency of the particular compound administered. Such variables will readily be accounted for by those of ordinary skill in the clinical art. In particular, dosages will be adjusted upward or downward for each recipient based on the severity of the condition to be treated and accessibility of the target cells to the pharmaceutical formulations of the invention. Where possible, it will be preferable to administer the pharmaceutical formulations of the

invention locally at the site of the target cells; e.g., into inflamed skin or by infusion to another organ of the host. Thus, dosages will also vary depending on the route of administration and the extent to which the formulations of the invention are expected to reach target cells before dilution or clearance of the formulation.

Taking these factors into account, acceptable CSAID and JNK activation cascade antagonist dosage ranges for each compound administered separately are about 0.1 to 80 mg/kg/day for oral and parenteral routes; about 0.1-150 mg/kg/day for topical routes and about 0.01-1 mg/kg/day for respiratory routes. Due to their

enhanced potency, bifunctional activation cascade antagonists are administered in lower dosages (e.g., at about a 10-40% lower dosage). Those of ordinary skill in the clinical arts will be able to determine medically sound dosing schedules for patients with particular presenting conditions, taking into account the severity of the condition, the patient's overall health, patient age and weight, and other clinically relevant factors. These dosages may be combined with other conventional pharmaceutical therapies for inflammation and fibrosis; e.g., corticosteroids.

Those of ordinary skill in the art will be familiar with, or can readily ascertain, the identity and clinical signs of specific inflammatory conditions, and can identify clinical signs of improvement therein (such as reductions in serum levels of TNF-α and improvement in clinical health) in addition to those exemplified herein.

V.

Methods for Identification of Therapeutically Effective Analogues

of Bifunctional activation cascade antagonists

Those of ordinary skill in the art will be familiar with means to develop analogues to the bifunctional activation cascade antagonists and constituents specifically described herein which, although not structurally identical thereto, may possess the same biological activity. Such compounds are within the scope of the invention and may be identified according to the protocols described below and in the Examples.

Though exposure of cells to the compounds of the invention under controlled conditions, the responsiveness of cells to inflammatory agents and intracellular mechanisms therefor can be investigated. This information will not only better elucidate the intracellular pathways responsible for cellular responses to particular stimuli, but will also aid in the identification of anti-inflammatory and anti-fibrosis therapeutic compounds.

To identify and select therapeutic compounds for use in treating conditions such as inflammation and fibrosis, lymphocytes, monocytes, neutrophils, intracellular components such as microsomes or immunologically naive animals are exposed to a PIC and the candidate therapeutic compound. Specifically, a control is incubated with a known amount of the inflammatory or fibroblast proliferation inducing agent. Treatment groups are exposed to the same amount of inflammatory or fibroblast proliferation inducing agent as well as aliquots of the candidate therapeutic compound. Inflammatory responses or fibroblast proliferation in each group are

detected by conventional means known to those of skill in the art (such as the assay steps described in the examples) and compared.

The invention having been fully described, examples illustrating its practice are set forth below. These examples should not, however, be considered to limit the scope of the invention, which is defined by the appended claims.

In the examples, the abbreviation "min." refers to minutes, "hrs" and "h" refer to hours, and measurement units (such as "ml") are referred to by standard abbreviations. "mp" refers to melting point.

EXAMPLE I

INHIBITION OF PIC ACTIVATION OF THE MAPK SYSTEM BY BIFUNCTIONAL ACTIVATION CASCADE INHIBITORS

As an *in vitro* model of MAPK system activation, peripheral blood monocyte cells (PBMC) were obtained from healthy human donors and stimulated with a PIC-sensitive-MAPK activator, namely lipopolysaccharide (LPS, Sigma). TNFα production was measured as the hallmark of MAPK cascade activation antagonism.

The PBMC were isolated from heparinized normal human blood by ficoll-histopaque density centrifugation. The cells were plated in 96 well plates with a JNK activation cascade antagonist (compound 54 or compound 37), a CSAID (SB 210313) or a bifunctional activation cascade antagonist composed of compound 54 or 37 and SB 210313. The latter was synthesized as described in Boehm, *et al.*, *J.Med.Chem.*, 39:3929-3937 (1997). SB210313 is 1-[3-(4-morpholinyl)propyl]-4-(4-flurophenyl)-5-(4-pyridyl) and is crystallized from acetone/hexane and stored as a dry powder at room temperature. The synthesized SB210313 compound tested is referred to below as I-105.

Each compound tested was added to the wells in equal volumes at concentrations of 10μm or 50μm. The plated cells were suspended in RPMI 1640 supplemented with 20% autologous plasma and glutamine and plated at a density of $5X10^5$ cells/ml. Cells were incubated in the plates for 60 minutes then each well received LPS at a final concentration of 10μg/ml. 18 hours later, 100μl of each well

was collected and assayed for TNFα content using a comercially available mouse anti-human TNFα antibody (Upstate Biotechnology, Inc.).

In the TNFα inhibition assay, compound 54 achieved 50% inhibition (IC_{50}) at 10μm. The IC_{50} for compound 37 was achieved at 20-25μm.. The IC_{50} for SB210313, one of the most potent CSAIDs, was achieved at 1.6 to 3.2μm. However, the maximal level of inhibition achieved by any of the compounds was only 70-80%.

In startingly contrast, the bifunctional activation cascade antagonist composed of compound 37 (the JNK activation cascade antagonist of *lesser* potency as compared to compound 54) and SB 210313 achieved IC_{50} in as little as 0.78 μm, a level more than merely additive of the activities of each constituent alone. Thus, the bifunctional activation cascade antagonists of the invention achieve synergy between the JNK activation cascade antagonist and CSAID constituents.

EXAMPLE II

EFFECT OF JNK ACTIVATION CASCADE ANTAGONISTS AND CSAIDs ON THE JNK ACTIVATION CASCADE

To demonstrate the probable efficacy of bifunctional activation cascade antagonists in antagonizing activation of JNK, the data below demonstrating the efficacy of JNK activation cascade antagonists in this context are provided.

MOLT-4 human lymphoblastoid cells were used as models of JNK activation in the presence of anisomycin.

MOLT-4 cells were preincubated with compound 54 to demonstrate the effect of compound 54 on JNK activation. The compound was dissolved in phosphate buffered saline and provided to the cells in concentrations of 50μm or 10μm. Incubations were made for intervals of 15, 30 or 60 minutes. Each incubated cell sample was activated with 50nM anisomycin for 30 minutes. Cells exposed only to PBS served as a control and, for comparison, one group of cell samples were exposed to compound 54 alone (without anisomycin) or anisomycin alone (without compound 54).

After the activation period, the MOLT-4 cell samples were washed three times and lysed. 100μg protein samples were used as reagents to measure enzyme activity in the MOLT-4 cell samples by a standard binding kinase assay, using GST c-Jun as the ligand and substrate.

For comparison, RAW mouse macrophages were stressed with a different stressor and the inhibitory potency of compound 54 vis-a-vis JNK activation was tested. The cells were pre-incubated with compound 54 for 60 minutes at increasing concentrations and activated with 1μg/ml LPS for 30 minutes. The cells were washed three times and lysed before testing.

The IC_{50}s for compound 54 varied from 10-50μm, depending on the stressor, the time of preincubation and the cell line studied. Specifically, the IC_{50} for compound 54 with respect to anisomycin-induced JNK activation was achieved in MOLT-4 cells at 10μm after 60 minutes of incubation. In contrast, virtually no

change in JNK activation is achieved by CSAIDs in similar models (see, e.g., Kumar, *et al., Biochem.Biophys.Res.Commun.*, 235:533-538 (1997)).

As demonstrated in Example I, the bifunctional activation cascade antagonists of the invention provide the activity of each antagonistic constituent in a synergistic manner. Thus, the bifunctional activation cascade antagonists provide the same activity of each constituent alone at greater potency.

EXAMPLE III

EFFECT OF CSAIDs AND JNK ACTIVATION CASCADE ANTAGONISTS ON THE p38 ACTIVATION CASCADE

To demonstrate the probable efficacy of bifunctional activation cascade antagonists in antagonizing activation of p38, the data below demonstrating the activity of JNK activation cascade antagonists and of CSAIDs in this context are provided.

Lymphocyte and monocyte extracts were prepared under similar conditions and were immunoprecipitated with affinity purified goat polyclonal antibody against the 20 amino acid N terminus of p38 (Santa Cruz Biotechnology), together with protein A SEPHAROSE™. The immunoprecipitates were washed, mixed with glutathione S-transferase-ATF2 SEPHAROSE™ beads as substrate, and resuspended in kinase buffer containing [γ-^{32}P]ATP. Samples were processed as described in the preceding Example, separated by SDS-PAGE and analyzed by autoradiography with Coomassie blue staining. Densitometry tracings of the 49 kDa GST-ATF2 band

showed that compound 54 does not block p38 kinase activation to an appreciable extent (only about 20% inhibition was achieved at 100μm).

In contrast, similar assays have demonstrated that CSAIDs inhibit activation of p38, but not of other kinases (see, e.g., Wilson, *et al., Chem.Biol.,* 4:423-431 (1997)).

These data, and those set forth in Example II, demonstrate that the JNK activation cascade antagonist and CSAID constituents of the bifunctional activation cascade antagonists of the invention antagonize activation of different MAPK pathways in a non-overlapping manner.

As demonstrated in Example I, the bifunctional activation cascade antagonists of the invention provide the activity of each antagonistic constituent in a synergistic manner. Thus, the bifunctional activation cascade antagonists provide the same activity of each constituent alone at greater potency.

EXAMPLE IV

INHIBITION OF CELL SENESCENCE AFTER SERUM DEPRIVATION IN SERUM-DEPENDENT CELLS BY JNK ACTIVATION CASCADE ANTAGONISTS

To demonstrate the probable efficacy of bifunctional activation cascade antagonists in inhibiting cell senescence, the data below demonstrating the efficacy of JNK activation cascade antagonists in this context are provided.

Many cell types are dependent upon serum factors for growth. Thus, deprivation of such cells of serum provides a model for assessment of compounds to modulate cell responses to intracellular ceramide-mediated signal transduction. In particular, withdrawal of serum from serum-dependent cell cultures produces increased intracellular levels of endogenous ceramide and may also increase intracellular levels of endogenous diacyl glycerol (see, e.g., Jayadev, *et al.*, *J.Biol.Chem.*, 270:2047-2052, 1995).

To evaluate the inhibitory effect of the compounds of the invention on ceramide-associated conditions *in vitro,* the serum withdrawal model was used. Specifically, 3T3 fibroblast cells were seeded in 96 well microtiter plates in DMEM in the presence of 10% fetal bovine serum. The cells were incubated to 90% confluence.

The medium was removed, the cells washed and reincubated in serum-free DMEM. Compound no. 37 and cell permeable ceramide were added to the wells at concentrations of, respectively, 0, 4, 40 or 400 μM compound no. 37 and 0, 5 or 10 μM of ceramide. After 24 hrs. incubation, 0.5 μCi of [^{3}H] thymidine was added to

each well for 2 hrs. DNA synthesis in the tested cell population was assessed by conventional techniques for detection of [^{3}H] thymidine incorporation. The results of this assay are indicated in FIGURE 1 and establish the cell senescence inhibitory efficacy of the inventive compounds (as represented by compound no. 37).

As demonstrated in Example I, the bifunctional activation cascade antagonists of the invention provide the activity of each antagonistic constituent in a synergistic manner. Thus, the bifunctional activation cascade antagonists provide the same activity of each constituent alone at greater potency.

EXAMPLE V

INHIBITION OF CELL APOPTOSIS AFTER CD95 STIMULATION BY JNK ACTIVATION CASCADE ANTAGONISTS

To demonstrate the probable efficacy of bifunctional activation cascade antagonists in inhibiting cell apoptosis, the data below demonstrating the efficacy of JNK activation cascade antagonists in this context are provided.

Engagement of cell surface receptor CD95 (also known as Fas/Apo-1 antigen) triggers cell apoptosis. DX2 is a functional anti-FAS (CD95) antibody which will, on binding of CD95, activate the Smase catalysis of sphingomyelin hydrolysis and production of ceramide (see, re DX2, Cifone, *et al., J.Exp.Med,* 177:1547-1552, 1993, the disclosure of which is incorporated herein by reference for use in accessing the DX2 antibody). Thus, binding of CD95 is a model for induction of apoptosis via the sphingomyelin signal transduction pathway.

To assess the inhibitory effect of the compounds of the invention on ceramide-mediated cell apoptosis, human T lymphoblasts (Jurkat) were suspended at $2x10^6$ cells per ml in RPMI-1640 supplemented with insulin, transferrin, selenium and glutamine. After incubation for 2 hrs. at room temperature with either compound no. 37, compound no. 6, pentoxifylline or a control compound (Ro-1724), 25 ng/ml of anti-FAS antibody was added to each suspension. After another 2 hrs., cell apoptosis was measured as a function of the number of cells (counted by hemocytometer) that excluded the vital dye erythrosin B. The results of the experiment are shown in FIGURE 2 and establish the apoptosis inhibitory efficacy of the compounds of the invention (as represented by compounds nos. 6 and 37, particularly the latter).

As demonstrated in Example I, the bifunctional activation cascade antagonists of the invention provide the activity of each antagonistic constituent in a synergistic manner. Thus, the bifunctional activation cascade antagonists provide the same activity of each constituent alone at greater potency.

EXAMPLE VI

INHIBITION OF THE ACTIVITY OF CERAMIDE ACTIVATED PROTEIN KINASE BY JNK ACTIVATION CASCADE ANTAGONISTS

To demonstrate the probable efficacy of bifunctional activation cascade antagonists in inhibiting activation of ceramide activated protein-kinase activation (at a site recognized by MAPK kinases), the data below demonstrating the efficacy of JNK activation cascade antagonists in this context are provided.

Ceramide-activated protein kinase (CaPK) is a 97 kDa protein which is exclusively membrane-bound and is believed to serve a role in the sphingomyelin signal transduction pathway. In particular, CaPK is believed to mediate phosphorylation of a peptide derived from the amino acid sequence surrounding Thr^{669} of the epidermal

growth factor receptor (i.e., amino acids 663-681). This site is also recognized by MAPK kinases. Thus, the effect of the JNK activation cascade antagonists on CaPK

activity in cells is indicative of the effect that the compounds exert on signal transduction in the sphingomyelin pathway.

To that end, Jurkat cells were suspended at $2x10^6$ cells per ml in RPMI-1640 medium. After incubation for 2 hrs., either compound 37, 20 μM of ceramide or 25 ng/ml of anti-FAS antibody DX2 were added to each suspension and incubated for 15 mins. After centrifugation and washing, the cells were separately homogenized in a dounce homogenizer.

Ceramide kinase levels in each test sample were assayed as described by Liu, *et al., J.Biol.Chem.*, 269:3047-3052, 1994 (the disclosure of which is incorporated herein for reference and use in assaying ceramide kinase). Briefly, the membrane fraction was isolated from each test sample of treated cell homogenate by ultracentrifugation and run on a 10% PAGE gel. The gel was washed with guanidine-HCL, and renatured in HEPES buffer. Then [^{32}P]-ATP was added to the gel and left there for 10 mins. Thereafter, the gel was extensively washed with 5% TCA. Autophos-phorylated kinase was detected by autoradiography.

The results of this assay are indicated in FIGURE 3, and establish the CaPK inhibitory efficacy of the compounds of the invention (as represented by compound 37).

As demonstrated in Example I, the bifunctional activation cascade antagonists of the invention provide the activity of each antagonistic constituent in a

synergistic manner. Thus, the bifunctional activation cascade antagonists provide the same activity of each constituent alone at greater potency.

EXAMPLE VII

ABSORBANCE OF UVB RADIATION

BY JNK ACTIVATION CASCADE ANTAGONISTS

To demonstrate the probable efficacy of bifunctional activation cascade antagonists in inhibiting absorbance of UVB radiation, the data below demonstrating the efficacy of JNK activation cascade antagonists in this context are provided.

Radiation (particularly in the UVB wavelength) is a major cause of skin damage (including apoptosis) in humans. As indicated elsewhere above, the sphingomyelin signal transduction pathway is believed to be involved in at least the early stages of development of radiation induced dermatoses (including radiation dermatitis, sunburn and UVB induced immune suppression from radiation damage to Langerhans cells in the skin- see, e.g., Haimovitz-Friedman, *et al., J.Exp.Med.,* 180:525-535, 1994 (cellular responses to ionizing radiation); and, Kurimoto and Streilein, *J.Immunol.,* 145:3072-3078, 1992 (cutnaceous iinmune suppression from UVB exposure)). Thus, a compound which will inhibit cell responses to stimulus of the sphingomyelin signal transduction pathway by radiation and can be administered topically at the site of exposure would be of great benefit in retarding the damage associated with radiation exposure (e.g., through exposure to sunlight or radiation).

To assess the radiation absorbing abilities of the compounds of the invention, the ultraviolet spectra of compounds of the invention (nos. 6 and 37, alone, in combination and as 8-oxo derivatives) were evaluated and compared to those of a commercially available sunscreen additive (PABA) and isoquinoline. The spectra were identified using a KONTRON analytical instrument. As indicated in FIGURE 4,

the compounds of the invention (as represented by compounds nos. 6 and 37) absorbed through most of the UVB region, indicating efficacy in absorbing radiation. Surprisingly, a mixture of compound nos. 6 and 37 proved to absorb throughout the UVB region. Thus, given the somewhat greater absorbance characteristics of compound 37 vis-a-vis compound 6, it can be reasonably expected that mixtures of the two in ratios of 1:1 or greater (favoring compound 37) will have substantial synergistic efficacy in absorbing radiation and retarding its effects on cells.

EXAMPLE VIII

INHIBITION OF TNF-α PRODUCTION BY JNK ACTIVATION CASCADE ANTAGONISTS(OTHER THAN COMPOUND 54)

To demonstrate the probable efficacy of bifunctional activation cascade antagonists in inhibiting TNFα production, the data below demonstrating the efficacy of JNK activation cascade antagonists in this context are provided.

As shown in FIGURE 5(a), compounds of the invention having N-1 chain lengths from 2-5 carbons are especially useful in inhibiting TNF-α production *in vitro,* while N-1 chain lengths of about 4 carbons (with a terminal ester) appear to be optimal in this respect (as compared to a control compound; FIGURE 5(b)). Further, the esterified compounds were significantly more effective inhibitors of TNF-α production than their carboxylic counterparts. These data were obtained as follows:

Peripheral blood mononuclear cells were isolated from normal human blood on Hypaque-Ficoll density gradients. A portion of the isolated cells were further purified by adherence to gelatin coated flasks.

100 µl aliquots of monocytes were placed onto 96 well microtiter plates at a density of $5x10^5$ cells/ml in RPMI-1640 medium containing 10% fetal bovine serum. After incubation for 24 hrs., various concentrations of the test compounds (FIGURE 10) were added to the plated cells in a volume of 100 µl and incubated for 1 hr. After incubation, 1 µg/ml of LPS was added to each well.

18 hrs. after exposure of the plated cells to LPS, 100 µl of medium was collected from each well and assayed (by ELISA) for release of TNF-α, using recombinant human TNF as a standard. The sensitivity of the assay ranged from 10-100 pg/ml.

As demonstrated in Example I, the bifunctional activation cascade antagonists of the invention provide the activity of each antagonistic constituent in a synergistic manner. Thus, the bifunctional activation cascade antagonists provide the same activity of each constituent alone at greater potency.

EXAMPLE IX

IN VIVO AND *IN VITRO* LEUKOPENIA IN RESPONSE TO LPS AND INHIBITION OF SAME BY JNK ACTIVATION CASCADE ANTAGONISTS

To demonstrate the probable efficacy of bifunctional activation cascade antagonists in inhibiting *in vivo* leukopenia, the data below demonstrating the efficacy of JNK activation cascade antagonists in this context are provided.

As shown in FIGURES 6 through 10 and Table 1, the compounds of the invention effectively reduce cellular response to LPS, a known inducer of TNF-α

production. In the presence of ceramide, the inhibitory activity of the compounds of the invention on LPS induced leukopenia (a phenomenon dependent on TNF-α induced surface expression of the P-selection class of adhesion molecules) was enhanced (FIGURE 6). However, the inhibitory activity of the compounds of the invention was essentially unaffected by diacylglycerol (FIGURE 7), indicating that the mode of action of the compounds of the invention are not dependent on hydrolysis of phosphatidic acid. These data were obtained as follows:

The leukopenia inhibitory capacity of the test compounds was determined by intraperitoneal administration of 0.5 μg of LPS in saline to ICR female mice (age 6-8 weeks; weight 19-23 g). One hour before receiving the LPS, the mice received the test compound by intraperitoneal injection at a dose of 50 mg/kg (in isotonic saline). Two hours after injection of LPS, 200 μl of blood was collected from each mouse into a

heparinized tube and the total count of nucleated cells determined in a hemocytometer.

Calcium independent protein kinase activity was measured, using a 1% triton X-100 extract of Jurkat cells ($5X10^8$/ml). The reaction mixture consisted of 20 mM Tris HCl pH 7.5, 20 mM $MgCl_2$, 20 μM ATP containing 200,000 cpm [γ32P] ATP, and 50 μM Myelin Basic Protein. The extract was pre-incubated with A) compound 37 B) compound 37 with or without 10 μM ceramide C) ceramide or D) dihydro ceramide for 15 minutes, followed by addition of substrate and ATP, and incubation at 30°C for 5 minutes. The total count of nucleated cells was measured in

a hemocytometer (FIGURE 8). The same protocol was followed to obtain the results shown in FIGURE 7 (with the addition of diacyl glycerol to some of the test mixtures).

An isoquinoline compound of the invention (compound 52) was also tested *in vitro* for its inhibitory efficacy with respect to LPS induced TNF-α production in human cells.

Human macrophages were cultured in 96 well microtiter plates and incubated with LPS. Aliquots of the stimulated cells were then incubated with, respectively, 0.1, 1, 10, 100 or 1000 μM of compound 52, compound 37 and a commercially available isoquinoline (6,7-dimethoxy-1(2H)-isoquinoline from Aldrich Chemical; labelled S52-626-6 in FIGURE 9) which, like the compounds of the invention, has an oxygen *ortho* to a ring nitrogen but, *unlike* the compounds of the invention, lacks a side chain

substituent as described above. The inhibitory efficacy of each compound was measured as a function of TNF-α reduction in pg/ml. The results of the experiment are indicated in FIGURE 11 and establish that the compounds of the invention (represented by compound 52 and compound 37) have inhibitory efficacy with respect to reduction in LPS induced TNF-α production by human cells. Other isoquinolines tested did not exert inhibitory activity in the absence of the side chain substituents added according to the invention.

These experiments were repeated in human lymphocytes. The protective abilities of the compounds tested is demonstrated by the data in FIGURES 12 and 13.

As demonstrated in Example I, the bifunctional activation cascade antagonists of the invention provide the activity of each antagonistic constituent in a synergistic manner. Thus, the bifunctional activation cascade antagonists provide the same activity of each constituent alone at greater potency.

EXAMPLE X

IN VIVO ANTI-INFLAMMATORY EFFICACY OF CSAIDs

The effects of CSAIDs on inflammation in an *in vivo* model of arthritis have been previously demonstrated. The following references are relevant in this regard and are incorporated herein for ease of reference by those in the art:

Boehm, *et al.*, *J.Med.Chem.*, 39:3929-3937 (1996) (SB 210313)

Badger, *et al.*, *J.Pharmacol.Exp.Ther.*, 279:1453-1461 (1996) (SB 203580)

As demonstrated in Example I, the bifunctional activation cascade antagonists of the invention provide the activity of each antagonistic constituent in a synergistic manner. Thus, the bifunctional activation cascade antagonists provide the same activity of each constituent alone at greater potency.

EXAMPLE XI

FIBROBLAST PROLIFERATION IN RESPONSE TO LPS AND INHIBITION OF SAME BY JNK ACTIVATION CASCADE ANTAGONISTS

To demonstrate the probable efficacy of bifunctional activation cascade antagonists in inhibiting fibrosis, the data below demonstrating the efficacy of JNK activation cascade antagonists in this context are provided.

As shown in FIGURE 10, PDGF induced fibroblast proliferation was selectively inhibited by the compounds of the invention. In addition, the compounds were shown not to be cytostatic or cytotoxic, insofar as they did not alter EGF-triggered mitogenesis in the cells tested (FIGURE 11).

These data were obtained as follows:

Mouse fibroblast line 3T3 cells (American Type Culture Collection #CCL 92) were seeded into 96 well plates in complete medium and allowed to grow to confluence. The medium was then replaced with medium-free serum and the cells incubated for 24 hrs.

The test compounds were then incubated with the cells for 1 hr before addition of 5 ng/ml human PDGF or EGF was added to each well. After another 24 hrs, 1 μCi of [^{3}H]-thymidine was added to each well. 4 hrs later the cells were harvested onto glass fiber filters and the cellular incorporation of [^{3}H]-thymidine was measured by liquid scintillation counting (FIGURES 10 and 11).

As demonstrated in Example I, the bifunctional activation cascade antagonists of the invention provide the activity of each antagonistic constituent in a synergistic manner. Thus, the bifunctional activation cascade antagonists provide the same activity of each constituent alone at greater potency.

EXAMPLE XII

SYNTHESIS OF COMPOUNDS 2, 4-8 AND 10-13

General Alkylation Procedure for Compounds **4-8, 10, 11** (Method A):

Theobromine or 8-bromotheobromine (2 mmol) was combined with anhydrous K_2CO_3 (2.5 mmol) and dry DMF (15 mL) and the mixture was brought to 75°C. The appropriate alkyl halide (2.5 mmol) was added and the mixture was stirred at 75°C for 2-18 h. The reaction mixture was cooled, poured into water (125 mL) and extracted with ethyl acetate (2 x 75 mL). The organic layer was dried over magnesium sulfate and evaporated to yield a colorless oil or white solid which was triturated with ethyl ether. The resulting solid, often analytically pure, may be purified further if desired by crystallization from a small amount of ethanol. Yields 58-89%. Compounds **15-17, 31, 36-38, 41, 43, 47,** and **48** (described below) were prepared by this same procedure only using the appropriate precursors in place of theobromine.

General Thiation Procedure for Compounds 12 and 13:

The 8-bromoxanthine **10** or **11** (0.25 mmol) was suspended in anhydrous ethanol (10 mL) and heated to reflux. $NaSH.(H_2O)_x$ (2.5 mmol) was added and the mixture became clear, green almost immediately. The mixture was stirred under reflux for 30 min, cooled and evaporated onto silica gel. Flash column chromatography using 5-7% MeOH in CH_2Cl_2 provided a 63% and 75% yield of **12** and **13**, respectively as white solids. Note: Compound **13** was found by ^1H NMR to be the ethyl ester due to transesterification under the reaction conditions.

^{1}H NMR spectra and elemental analyses or exact mass data were consistent with the assigned structures.

EXAMPLE XIII

SYNTHESIS OF COMPOUNDS 24, 25, 31 AND INTERMEDIATES

General Procedure for C-Nitrosation of Pyrimidines (Compounds **18-20**, **27**, and **32**):

The pyrimidine (15 mmol) was suspended in N HCl (30 mL) and an aqueous solution of sodium nitrite (20 mmol in 10 mL) was dripped in with stirring over 10 min. The suspension went from off-white to purple almost immediately. Stirring was continued for !h, pH adjusted to 5 with ammonia water and the purple solid product collected to provide 75-90% yield after drying. The characteristic lack of the C-5 proton in the ^{1}H NMR was evident for each compound (Table 2).

General Procedure for the Reduction of 5-Nitroso to 5-Amino Pyrimidines (Compounds **21-23, 28, and 33**):

The 5-nitrosopyrimidine (15 mmol) was suspended in water (50 mL) and heated to 80-90°C. With stirring, sodium hydrosulfite (45 mmol) was added in portions over 5 min. The color quickly changed from purple to light green and stirring was continued an additional 10 min. The mixture was cooled in ice and filtered. The filtered solid was washed with cold water, EtOH and Et_2O to provide the orthodiamine in 70-88% yield as a tan to pale green solid.

Synthesis of 1-n-Hexyl-3-methyluric acid intermediate (**24**):

The nitrosopyrimidine **19** (270 mg, 1.06 mmol) was dissolved in ethanol (20 mL) with warming and palladium on carbon (75 mg, 10%) was added under argon. Hydrogenation was performed at room temperature and 15 psi for 2 h, filtered to remove catalyst and evaporated to dryness. The residue was combined with urea (600 mg, 10 mmol) and heated neat on the hot plate with stirring. The temperature reached 140°C which produced a clear melt and was maintained for about 10 min. with additional urea added (1 g). Upon cooling the melt solidified and was dissolved in n NaOH (25 mL) and boiled with decolorizing carbon for 10 min., filtered and acidified to pH 3-4 while hot. The resulting precipitate was collected after cooling and washed with water and dried to yield 160 mg (57%) of **24** as an off-white solid with the following characteristics: mp >290°C dec. ^{1}H NMR (500 MHZ, DMSO-d_6) δ 11.80 and 10.73 (2s, 2H, N-7 H, N-9 H), 3.78 (t, 2H, N-CH_2), 3.30 (s, 3H, N-CH_3, under H_2O signal), 1.48 (m, 2H, 2'CH_2), 1.24 (m, 6H, 3', 4', 5' CH_2), 0.85 (t, 3H, CH_3). Analysis: $C_{12}H_{18}N_4O_3$ (C, H, N; Table 2).

Synthesis of **3-Methyl-8-thiouric acid (25)** intermediate:

The pyrimidinediamine **33** (100 mg, 0.63 mmol) was combined with potassium ethyl xanthate (810 mg, 5 mmol) and DMF (10 mL) and heated at 100°C. The suspension became green almost immediately and reaction was complete after 30 min. by TLC. After a total reaction time of 1 h, the mixture was cooled, filtered and washed with Et_2O, dried to yield an off-white solid (310 mg) which presumably contained the unreacted potassium ethyl xanthate and the potassium salt of the desired

product. The solid was suspended in water (5 mL) and heated to dissolve. Glacial acetic acid was added to pH 5 and a vigorous effervescence was noted. A white solid formed which was filtered warm and washed with water, then ethanol and dried to yield 99 mg (79%) of the title compound. ^{1}H NMR (DMSO-d_6) δ 13.40, 12.92 and 11.80 (3br s, 3H, NHs), 3.28 (s, 3H, CH_3). Analysis: $C_6H_6N_4O_2S$ (C, H, N; Table 2).

Synthesis of **3-n-Propylxanthine (29)** intermediate:

The pyrimidinediamine **28** (750 mg) was combined with diethoxymethyl acetate (7 mL) and heated at 80°C for 2 h. The mixture was evaporated to dryness and water (5 mL) was added and the mixture heated for 20 min. to near boiling. The resulting solution was then allowed to evaporate slowly to yield off-white crystals. Yield 680 mg (86%); mp 282-284°C, Lit.[15] 291-292°C.

EXAMPLE XIV

SYNTHESIS OF COMPOUNDS 36-39, 41 AND 43 AND INTERMEDIATES

General Procedure for Ring Closure of Pyrimidine-diamines to Pteridines:

The orthodiamine **28** or **33** (2 mmol) was suspended in water (20 mL) and heated to above 70°C before a solution of glyoxal-sodium bisulfite addition product (10 mmol in 25 mL water) was added with stirring. The pale green suspension slowly became light amber and clear. After heating 5 min TLC indicated reaction was complete. The mixture was cooled and extracted with ethyl acetate (5 x 40 mL), dried over $MgSO_4$ and evaporated to yield the 1-methyl (**34**) or 1-n-propylpteridine (**35**) in

71 and 78 %, respectively. ^{1}H NMR showed the appearance of two aromatic signals at about 8.74 and 8.55 as doublets (J = 2.5 Hz) for both compounds.

Synthesis of **6,7-Diethyl-1-methylpteridine-2,4-dione (40)** intermediate:

Compound **33** (200 mg, 1.27 mmol) was suspended in acetonitrile (5 mL) and 3,4-hexanedione (185 μL, 1.52 mmol) was added. The mixture was heated at 70°C for 15 min with minimal product formation due to insolubility of **33**. Therefore DMF (3 mL) and water (3 mL) were added and the temperature was raised to 100°C. After 90 min total reaction time the mixture was cooled and poured into water (100 mL) and extracted with ethyl acetate (3 x 75 mL). The organic layer was dried over $MgSO_4$ and evaporated to provide the colorless crystalline product. Yield 240 mg (81%); mp 218-222°C; ^{1}H NMR (DMSO-d_6) δ 11.78 (br s, 1H, NH), 3.46 (s, 3H, NCH_3), 2.95 and 2.93 (2q, 4H, $2CH_2$ of ethyls), 1.28 and 1.23 (2t, 6H, $2CH_3$ of ethyls). Analysis: $C_{11}H_{14}N_4O_2$ (C, H, N; Table 2).

Synthesis of **1-Methyl-6-phenylpteridine-2,4-dione (42)** intermediate:

The nitrosopyrimidine **32** (220 mg, 1.28 mmol) was mixed thoroughly with phenethyl amine hydrochloride (1.5 g, 9.5 mmol) and heated in an open beaker on the hot plate. After a few minutes at about 160°C the purple reaction mixture fused to a brown paste. TLC indicated many products so sulfolane (1 mL) was added and heat was continued for 15 min. The reaction mixture was heated in water (10 mL) and then diluted 50 mL in water and extracted with ethyl acetate (2 x 50 mL), the organic layer dried over $MgSO_4$ and then concentrated. The residue was flash

chromatographed on silica gel using 4% MeOH in CH_2Cl_2. Yield 75 mg (23%) of **42** as a pale yellow-orange solid. mp >307°C dec.; ^{1}H NMR (500 MHZ, DMSO-d_6) δ 11.95 (br s, 1H, NH), 9.37 (s, 1H, C-7 H), 8.17 (m, 2H, 2',6' phenyl), 7.55 (m, 3H, 3',4',5' phenyl), 3.51 (s, 3H, NCH_3). Anal. $C_{13}H_{10}N_4O_2$ (C, H, N).

EXAMPLE XV

SYNTHESIS OF COMPOUNDS 44, 47 AND 48

General Method for Ring Closure of Pyrimidines to Thiadiazolo-pyrimidines (Compounds **44-46**):

The orthodiamine **23, 27,** or **32** (2.3 mmol) was suspended in dry acetonitrile (5 mL) and dry pyridine (1.5 mL) was added. Thionyl chloride (1 mL, 13.7 mmol) was added quickly and the mixture, which became clear and darkened, was heated at 60°C for 10 min. The mixture was then cooled and poured into n HCl (40 mL) with stirring. The resulting yellow solution was extracted with ethyl acetate (3 x 40 mL), dried over $MgSO_4$ and evaporated to yield a pale yellow solid which was triturated with ether. Yield 65-74%.

Alkylation of these intermediates yielded the disubstituted products **47** and **48**.

EXAMPLE XVI

SYNTHESIS OF COMPOUNDS 50 AND 52

Ethyl 4-[(2-methylamino)benzoyl]aminobutanoate (51):

A mixture of N-methylisatoic anhydride (3.5 g, 19.8 mmol) was combined with 4-aminobutyric acid (2.5 g, 24.3 mmol) in dry DMF (50 mL) and heated at 100°C for 2 h. TLC indicated reaction to be complete and the DMF was removed in vacuo. The residue was used directly for esterification which was accomplished by dissolving the residue in 100% ethanol (50 mL) and adding chlorotrimethyl silane (2.5 mL, 20 mmol). The mixture was heated at 65°C for 6 h and then evaporated to yield

a brown syrup. Crude yield 87% from isatoic anhydride. A small sample was purified for characterization and biological testing by preparative TLC using 7% MeOH in CH_2Cl_2. The remainder of the material was used directly for preparation of compound **52**. Analysis: $C_{14}H_{20}N_2O_3$ (C, H, N; Table 2).

Ethyl 1-Methyl-1,4-dihydro-2,4-dioxo-3(2*H*)-quinazolinebutanoate (52):

The residue from **51** was combined with ethyl chloroformate (10 mL) and heated at 90°C for 1 h. The mixture was cooled and poured into saturated aqueous sodium bicarbonate (50 mL) with stirring and after 10 min extracted with ethyl acetate (2 x 75 mL). The organic layer was dried over $MgSO_4$ and evaporated to yield a brown syrup. The crude product was flash chromatographed on silica using 3% MeOH in CH_2Cl_2 to yield g (%) of **52** as a thick oil. ^1H NMR (500 MHZ, DMSO-d_6) δ 7.27-7.42 (2m, 4H, C-5,6,7,8), 4.04 (t, 2H, CH_2 of ethyl), 3.88 (m, 2H, NCH_2), 3.11 (s, 3H, NCH_3), 2.33 (t, 2H, 2'CH_2), 1.71 (m, 2H, 3'CH_2). Analysis: $C_{15}H_{18}N_2O_4$ (C, H, N).

EXAMPLE XVII

SYNTHESIS OF HIGHLY WATER SOLUBLE DERIVATIVES

Acid Hydrolysis of Compound 53.

Compound **53** (1.63 g, 5.1 mmol) was combined with n HCl (30 mL) and was heated at boiling for 75 min. The mixture was cooled and the resulting solid which formed was filtered, washed with cold water, and dried to yield 1.5 g

(quantitative conversion) of the free carboxylic acid of suitable purity for esterification (as judged by TLC).

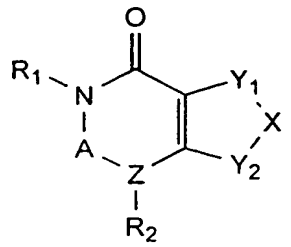
Esterification to form morpholinoethyl ester Compound 54.

The carboxylic acid obtained above in this Example (1.0 g, 3.4 mmol) was dissolved in dichloromethane (25 mL) with warming and then thionyl chloride (1 mL, 13.7 mmol) was added followed by 3 drops of DMF. After a few minutes a white solid precipitated (acid chloride intermediate), but the reaction mixture was allowed to stir at room temperature overnight. The mixture was evaporated to remove excess thionyl chloride and the residue was suspended in dry acetonitrile (25 mL). To this mixture was added morpholinoethanol (1.24 mL, 10.2 mmol) and heated at 80°C for 5 minutes. The solid suspension became clear almost immediately and after cooling, the reaction mixture was evaporated onto silica gel and loaded on a flash silica gel column and eluted with methanol - dichloromethane 5/95. The fractions of pure product were pooled and evaporated to yield an off-white residue which was dissolved in isopropyl alcohol (5 mL) and concentrated HCl (1 mL) was added. Upon concentration in vacuo, an off-white solid formed. Yield after drying 0.93 g (62%), mp 90°C. Proton NMR confirms structure assignment as the morpholino ethyl ester of Compound **53**: ^{1}H NMR (DMSO-d_6, 500 MHZ) δ 7.56 and 7.15 (2s, 2H, C-5 and C-8 aromatics), 7.35 and 6.55 (2d, 2H, C-3 and C-4 aromatics), 4.37 (m, 2H, C-1' of ester), 3.92 (m, 2H, C-4 of acyl), 3.86 and 3.84 (2s, 6H, OMe's), 3.95, 3.45, 3.10 (3m, 8H, morpholino and C-2'), 2.38 (t, 2H, C-2 of acyl), 1.92 (m, 2H, C-3 of acyl).

The invention having been fully described, modifications thereof may be apparent to those of ordinary skill in the art. Such modifications are within the scope of the invention as defined by the appended claims.

CLAIMS

1. A compound for antagonizing activation of c-Jun kinase and p38 comprised of a first constituent having the formula:



wherein:

R_1 is a terminally substituted normal alkyl having from 1 to 7 carbon atoms, a terminally substituted alkenyl having from 2 to 7 carbon atoms, a terminally substituted ether having from 2 to 6 carbon atoms, a terminally substituted secondary amine having from 2 to 6 carbon atoms, or substituted aryl having less than 8 carbons, where said terminal group is NH_2, substituted amino, acyloxy, SO_3H, PO_4H_2, NNO(OH), SO_2NH_2, $PO(OH)NH_2$, SO_2R or COOR, where R is H, an alkyl having from 1 to 4 carbon atoms, an alkenyl having from 1 to 4 carbon atoms, tetrazolyl, benzyl, or an alkylamino, where the alkyl group has from 1 to 4 carbon atoms and the amino is NH_2 or a substituted amino where the substituents

on the amino have 1 to 6 carbon atoms, one of which can be replaced by an oxygen atom or nitrogen atom;

Z is C, CH, or N;

R_2 is an alkyl, a cyclic alkyl, a heterocyclic alkyl, alkenyl, or aralkyl having less than 7 carbon atoms when Z is C, R_2 is a halogen, NO, amino, or substituted amino when Z is CH, or R_2 is H, an alkyl, a cyclic alkyl, a heterocyclic alkyl, alkenyl, or aralkyl having less than 7 carbon atoms when Z is N;

A is CO when Z is N, or CR_5 when Z is C or CH, where R_5 is H, an alkyl, a cyclic alkyl, a heterocyclic alkyl, alkenyl, aryl or aralkyl having less than 7 carbon atoms, OH, or an O-alkyl having from 1 to 5 carbon atoms and there is a double bond between Z and A when Z is C and a single bond between Z and A when Z is CH;

Y_1 is N, NR_6 or CR_6, where R_6 is H, NO, an amino, a substituted amino, an alkyl, a cyclic alkyl, a heterocyclic alkyl, alkenyl, or aralkyl having less than 7 carbon atoms;

Y_2 is N or CH; and

X is S when Y_1 and Y_2 are N, CR_7 when Y_1 is NR_6 where R_7 is H, OH, SH, Br, Cl, or I, or $=C(R_3)-C(R_4)=$ when Y_1 is N, CH or CR_6 and Y_2 is N or CH, where each of R_3 and R_4, independently, is H, an alkyl, a cyclic alkyl,

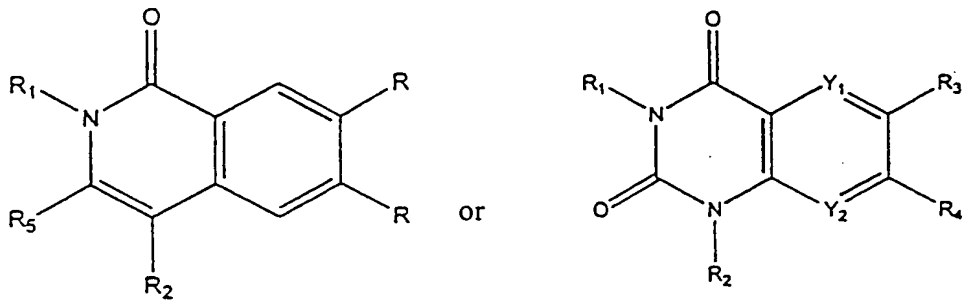
a heterocyclic alkyl, alkenyl, aryl, alkylcarboxyl, or aralkyl having less than 7 carbon atoms, SH, OH or an O-alkyl having from 1 to 5 carbon atoms,

or a salt thereof; and,

a second constituent consisting of a pyridinylimidazole conjugated to the first constituent;

wherein the first constituent selectively antagonizes c-Jun kinase activation and the second constituent selectively antagonizes p38 activation.

2. The compound according to Claim 1, wherein the first constituent has the formula:



wherein

R_1 is a terminally substituted normal alkyl having from 1 to 7 carbon atoms, a terminally substituted alkenyl having from 2 to 7 carbon atoms, a terminally substituted ether having from 2 to 6 carbon atoms, a terminally substituted secondary amine having from 2 to 6 carbon atoms, or substituted aryl having less than 8 carbons, where said terminal group is NH_2, substituted amino, acyloxy, SO_3H, PO_4H_2, NNO(OH), SO_2NH_2, $PO(OH)NH_2$, SO_2R or COOR where R is H, an alkyl having from 1 to 4 carbon atoms, an alkenyl having from 1 to 4 carbon atoms, tetrazolyl, benzyl, or an alkylamino, where the alkyl group has from 1 to 4 carbon atoms and the amino is NH_2 or a substituted amino where the substituents on the amino have 1 to 6 carbon atoms, one of which can be replaced by an oxygen atom or nitrogen atom;

R_2 is an alkyl, a cyclic alkyl, a heterocyclic alkyl, alkenyl, or aralkyl having less than 7 carbon atoms;

each of Y_1 and Y_2, independently, is N or CH;

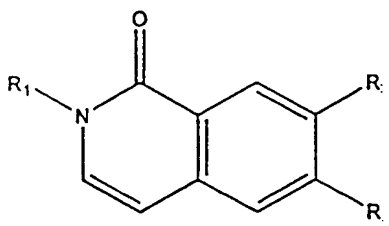
each of R_3 and R_4, independently, is H, SH, OH, an alkyl, a cyclic alkyl, a heterocyclic alkyl, alkenyl, aryl, alkylcarboxyl, or aralkyl having less than 7 carbon atoms, or OH or an O-alkyl having from 1 to 5 carbon atoms;

R_5, when present, is H, an alkyl, a cyclic alkyl, a heterocyclic alkyl, alkenyl, aryl or aralkyl having less than 7 carbon atoms, OH, or an O-alkyl having from 1 to 5 carbon atoms; and

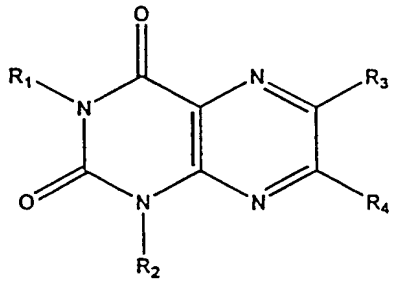
R_6 is NO, an amino, or a substituted amino;

or a salt thereof.

The compound of Claim 2 wherein the first constituent has the formula:



or



wherein

R_1 is a terminally substituted normal alkyl having from 1 to 7 carbon atoms, a terminally substituted alkenyl having from 2 to 7 carbon atoms, a terminally substituted ether having from 2 to 6 carbon atoms, a terminally substituted secondary amine having from 2 to 6 carbon atoms, or substituted aryl having less than 8 carbons, where said terminal group is NH_2, substituted amino, acyloxy, SO_3H, PO_4H_2, NNO(OH), SO_2NH_2, $PO(OH)NH_2$, SO_2R or COOR where R is H, an alkyl having from 1 to 4 carbon atoms, an alkenyl having from 1 to 4 carbon atoms, tetrazolyl, benzyl, or an alkylamino, where the alkyl group has from 1 to 4 carbon atoms and the amino is NH_2 or a substituted amino where the substituents on the amino have 1 to 6 carbon atoms, one of which can be replaced by an oxygen atom or nitrogen atom;

R_2, when present, is an alkyl, a cyclic alkyl, a heterocyclic alkyl, alkenyl, or aralkyl having less than 7 carbon atoms; and

each of R_3 and R_4, independently, is H, an alkyl, a cyclic alkyl, a heterocyclic alkyl, alkenyl, aryl, alkylcarboxyl, or aralkyl having less than 7 carbon atoms, or OH or an O-alkyl having from 1 to 5 carbon atoms;

or a salt thereof.

4. The compound of Claim 3, wherein the first constituent has the following substituents:

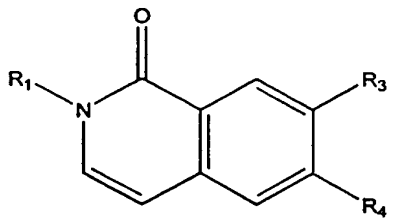
R_1 is a terminally substituted normal alkyl having from 1 to 7 carbon atoms, where said terminal group and is NH_2, substituted amino, or COOR, where R is H, an alkyl having from 1 to 4 carbon atoms, or an alkylamino, where the alkyl group has from 1 to 4 carbon atoms and the amino is a substituted amino where the substituents on the amino have 1 to 6 carbon atoms, one of which can be replaced by an oxygen atom or nitrogen atom;

R_2, when present, is an alkyl having less than 7 carbon atoms; and

each of R_3 and R_4, independently, is H, an alkyl, aryl, alkylcarboxyl, or aralkyl having less than 7 carbon atoms, or an O-alkyl having from 1 to 5 carbon atoms;

or a salt thereof.

5. The compound of Claim 4 wherein the first constituent has the formula:



wherein

R_1 is a terminally substituted normal alkyl having from 1 to 7 carbon atoms where said terminal group is COOR, where R is H, an alkyl having from 1 to 4 carbon atoms, or an alkylamino, where the alkyl group has from 1 to 4 carbon atoms and the amino is a morpholino group; and

each of R_3 and R_4, independently, is H, an alkyl having less than 7 carbon atoms, or an O-alkyl having from 1 to 5 carbon atoms;

or a salt thereof.

6. The compound of Claim 5, wherein the first constituent has the following substituents:

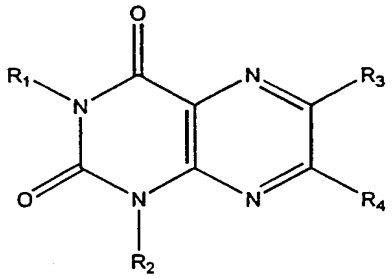
R_1 is a terminally substituted normal alkyl having from 1 to 4 carbon atoms where said terminal group is COOR where R is an N-morpholinoalkyl group; and,

each of R_3 and R_4, independently, is an O-alkyl having from 1 to 5 carbon atoms;

or a salt thereof.

7. The compound of Claim 6, wherein R_1 is $-(CH_2)_3COOR$ where R is an N-morpholinoethyl group, and R_3 and R_4 each are methoxy groups, or a salt thereof.

8. The compound of Claim 4, wherein the first constituent has the formula:



wherein

R_1 is a terminally substituted normal alkyl having from 1 to 7 carbon atoms where said terminal group is COOR, where R is H, an alkyl having from 1 to 4 carbon atoms, or an alkylamino, where the alkyl group has from 1 to 4 carbon atoms and the amino is a morpholino group;

R_2 is an alkyl, a cyclic alkyl, a heterocyclic alkyl, alkenyl, or aralkyl having less than 7 carbon atoms; and

each of R_3 and R_4, independently, is H, OH, SH, an alkyl, aryl, or aralkyl having less than 7 carbon atoms, or an O-alkyl having from 1 to 5 carbon atoms;

or a salt thereof.

9. The compound of Claim 8, wherein the first constituent has the following substituents:

R_1 is a terminally substituted normal alkyl having from 1 to 4 carbon atoms where said terminal group is COOR where R is an N-morpholinoalkyl group;

R_2 is an alkyl having less than 7 carbon atoms; and

each of R_3 and R_4, independently, is H, an alkyl, aryl, or alkylcarboxyl having less than 7 carbon atoms, or an O-alkyl having from 1 to 5 carbon atoms;

or a salt thereof.

10. The compound of Claim 9, wherein the first constituent has the following substituents:

R_1 is $-(CH_2)_3COOR$ where R is an N-morpholinoethyl group;

R_2 is an alkyl having less than 4 carbon atoms; and

each of R_3 and R_4, independently, is H, an alkyl having 1 to 3 carbon atoms, phenyl, or methylcarboxyl;

or a salt thereof.

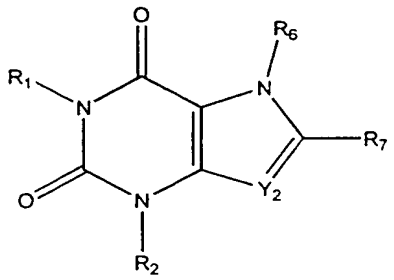
11. The compound of Claim 10, wherein the first constituent has the following substituents:

R_2 is $-(CH_2)_2CH_3$; and,

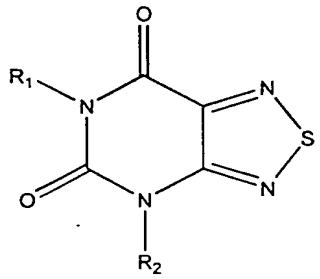
R_3 and R_4 each are H;

or a salt thereof.

12. The compound according to Claim 1 wherein the first constituent has the formula:



or



wherein

R_1 is a terminally substituted normal alkyl having from 1 to 7 carbon atoms, a terminally substituted alkenyl having from 2 to 7 carbon atoms, a terminally substituted ether having from 2 to 6 carbon atoms, a terminally substituted secondary amine having from 2 to 6 carbon atoms, or substituted aryl having less than 8 carbons, where said terminal group is NH_2, substituted amino, acyloxy, SO_3H, PO_4H_2, NNO(OH), SO_2NH_2,

$PO(OH)NH_2$, SO_2R or COOR, where R is H, an alkyl having from 1 to 4 carbon atoms, an alkenyl having from 1 to 4 carbon atoms, tetrazolyl, benzyl, or an alkylamino, where the alkyl group has from 1 to 4 carbon atoms and the amino is NH_2 or a substituted amino where the substituents on the amino have 1 to 6 carbon atoms, one of which can be replaced by an oxygen atom or nitrogen atom;

R_2 is H, an alkyl, a cyclic alkyl, a heterocyclic alkyl, alkenyl, or aralkyl having less than 7 carbon atoms;

Y_2 is N or CH;

R_6 is H, an alkyl, a cyclic alkyl, a heterocyclic alkyl, alkenyl, or aralkyl having less than 7 carbon atoms; and

R_7 is H, OH, SH, Br, Cl, or I;

or a salt thereof; and,

a second constituent consisting of a pyridinylimidazole;

wherein the first constituent selectively antagonizes c-Jun kinase activation and the second constituent selectively antagonizes p38 activation.

13. The compound of Claim 12, wherein the first constituent has the following substituents:

R_1 is a terminally substituted normal alkyl having from 1 to 7 carbon atoms, where said terminal group is NH_2, substituted amino, or COOR, where R is H, an alkyl having from 1 to 4 carbon atoms, or an alkylamino, where the alkyl group has from 1 to 4 carbon atoms and the amino is NH_2 or a substituted amino where the substituents on the amino have 1 to 6 carbon atoms, one of which can be replaced by an oxygen atom or nitrogen atom;

R_2 is H, or an alkyl having less than 7 carbon atoms;

Y_2 is N or CH;

R_6 is H, or an alkyl having less than 7 carbon atoms; and

R_7 is H, OH, SH, or Br;

or a salt thereof.

14. The compound of Claim 13, wherein R_1 is $(CH_2)_3COOR$ and R is an N-morpholinoethyl group, or a salt thereof.

15. The compound of Claim 1, wherein R_1 is $(CH_2)_3COOR$ and R is an N-morpholinoethyl group, or a salt thereof.

16. The compound according to Claim 1, wherein the second constituent is a cytokine suppressive anti-inflammatory drug.

17. The compound according to Claim 16, wherein the cytokine suppressive anti-inflammatory drug is selected from the group of such drugs comprising 1-[3-(4-morpholinyl)propyl]-4-(4-fluorophenyl-5-(4-pyridyl)imidazole, 2-(4-methylsulfinylphenyl)-3-(4-pyridyl)-6,7-dihydro-[5H]-pyrrolo[1,2-a]imidazole, 2-(4-methylthiophenyl)-3-(4-pyridyl)-6,7-dihydro-[5H]-pyrrolo[1,2-a]imidazole, 4-(4-fluorophenyl)-2-(4-methylsulfinyl)-5-(4-pyridyl)imidazole and 5-(4-pyridyl)-6(4-flurophenyl)-2,3-dihydroimidazole(2,1-b)thia zol.

18. The compound according to Claim 1, wherein the first and second constituents are conjugated to one another by a bond which is severable *in vivo*.

19. The compound according to Claim 18, wherein the bond is an amide, ester or azo bond.

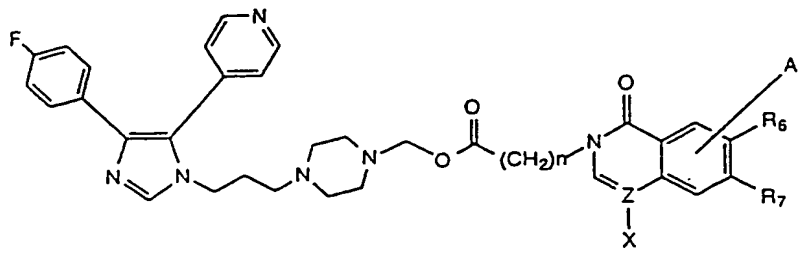
20. The compound according to Claim 19, wherein the bond is a tertiary N acyloxymethyl amide bond.

21. The compound according to Claim 1 mixed in a colloid.

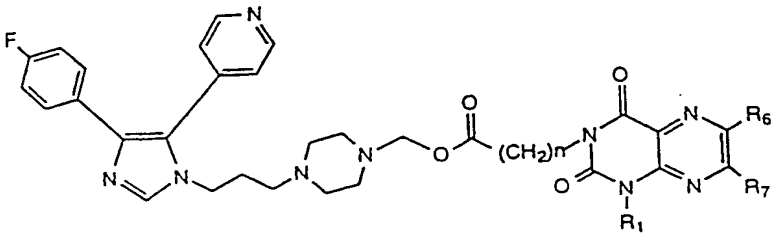
22. The compound according to Claim 21, wherein the colloid is a liposome.

23. A pharmaceutical composition comprising the compound of Claim 1 and a pharmaceutically acceptable carrier.

24. A compound for antagonizing activation of c-Jun kinase and p38 having the formula:



or



wherein

n is any number of carbon atoms from 1 to 7, O or N;

R_1, if present, is H, an alkyl, a cyclic alkyl, a heterocyclic alkyl, alkenyl, or aralkyl having less than 7 carbon atoms;

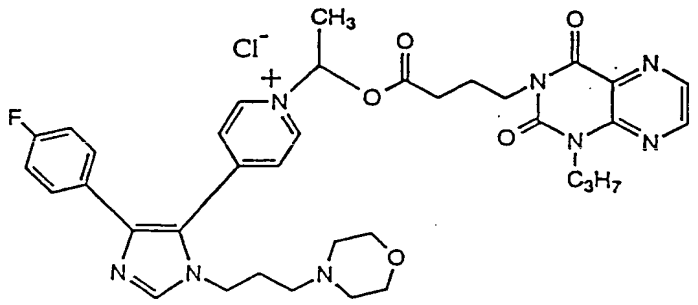
R_6 and R_7 are H, OH or OR_1, in any combination;

Z is N or C;

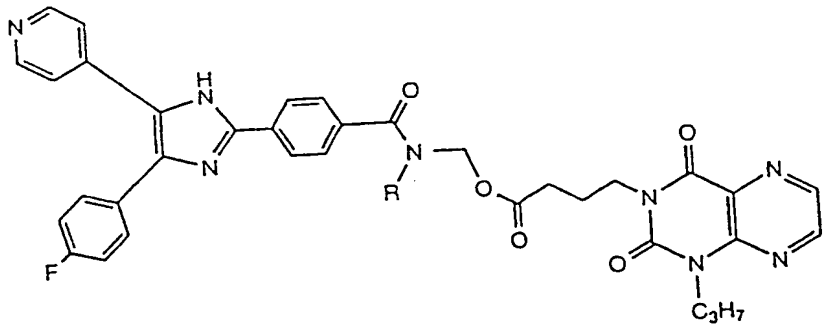
X, where Z is C, is H, halogen, N_3, NO, NH_2, NHR_1, $N(R_1)2$ or COR_1; and,

A is H, halogen, N_3, NO, NH_2, NHR_1, $N(R_1)2$ or COR_1.

25. A compound according to Claim 24 having the formula:



26. A compound according to Claim 24 having the formula:

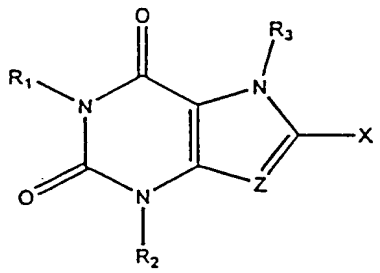


27. A pharmaceutically acceptable composition comprising the compound according to Claim 24 mixed in a colloid.

28. The compound according to Claim 27, wherein the colloid is a liposome.

29. A pharmaceutical composition comprising the compound of Claim 24 and a pharmaceutically acceptable carrier.

30. A compound comprised of a first constituent having the formula:



wherein

R_1 is $(CH_2)COO$-Et;

R_2 is CH_3;

R_3 is CH_3; and,

Z is N;

further comprised of a second constituent conjugated to the first constituent and consisting of a pyridinylimidazole which selectively antagonizes p38 activation.

31. The compound according to Claim 30, wherein the pyridinylimidazole is a cytokine suppressive anti-inflammatory drug.

32. The compound according to Claim 31, wherein the cytokine suppressive anti-inflammatory drug is selected from the group of such drugs comprising 1-[3-(4-morpholinyl)propyl]-4-(4-fluorophenyl-5-(4-pyridyl)imidazole, 2-(4-methylsulfinylphenyl)-3-(4-pyridyl)-6,7-dihydro-[5H]-pyrrolo[1,2-a]imidazole, 2-(4-methylthiophenyl)-3-(4-pyridyl)-6,7-dihydro-[5H]-pyrrolo[1,2-a]imidazole, 4-(4-fluorophenyl)-2-(4-methylsulfinyl)-5-(4-pyridyl)imidazole and 5-(4-pyridyl)-6(4-flurophenyl)-2,3-dihydroimidazole(2,1-b)thia zol.

33. The compound according to Claim 30, wherein the first and second constituents are conjugated to one another by a bond which is severable *in vivo*.

34. The compound according to Claim 33, wherein the bond is an amide, ester or azo bond.

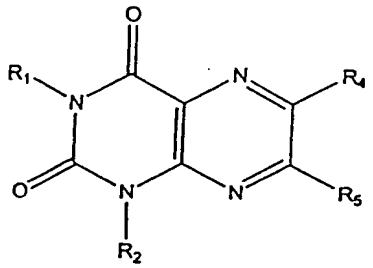
35. The compound according to Claim 34, wherein the bond is a tertiary N acyloxymethyl amide bond.

36. The compound according to Claim 30, wherein the first and second constituents are mixed in a colloid.

37. The compound according to Claim 36, wherein the colloid is a liposome.

38. A pharmaceutical composition comprising the compound of Claim 30 and a pharmaceutically acceptable carrier.

39. A compound comprised of a first constituent having the formula:



wherein

R_1 is $(CH_2)_3$ COO-Et;

R_2 is n-propyl;

R_4 is H; and

R_5 is H;

further comprised of a second constituent conjugated to the first constituent and consisting of a pyridinylimidazole which selectively antagonizes p38 activation.

40. The compound according to Claim 39, wherein the pyridinylimidazole is a cytokine suppressive anti-inflammatory drug.

41. The compound according to Claim 40, wherein the cytokine suppressive anti-inflammatory drug is selected from the group of such drugs comprising 1-[3-(4-

morpholinyl)propyl]-4-(4-fluorophenyl-5-(4-pyridyl)imidazole, 2-(4-methylsulfinylphenyl)-3-(4-pyridyl)-6,7-dihydro-[5H]-pyrrolo[1,2-a]imidazole, 2-(4-methylthiophenyl)-3-(4-pyridyl)-6,7-dihydro-[5H]-pyrrolo[1,2-a]imidazole, 4-(4-fluorophenyl)-2-(4-methylsulfinyl)-5-(4-pyridyl)imidazole and 5-(4-pyridyl)-6(4-flurophenyl)-2,3-dihydroimidazole(2,1-b)thia zol.

42. The compound according to Claim 39, wherein the first and second constituents are conjugated to one another by a bond which is severable *in vivo*.

43. The compound according to Claim 42, wherein the bond is an amide, ester or azo bond.

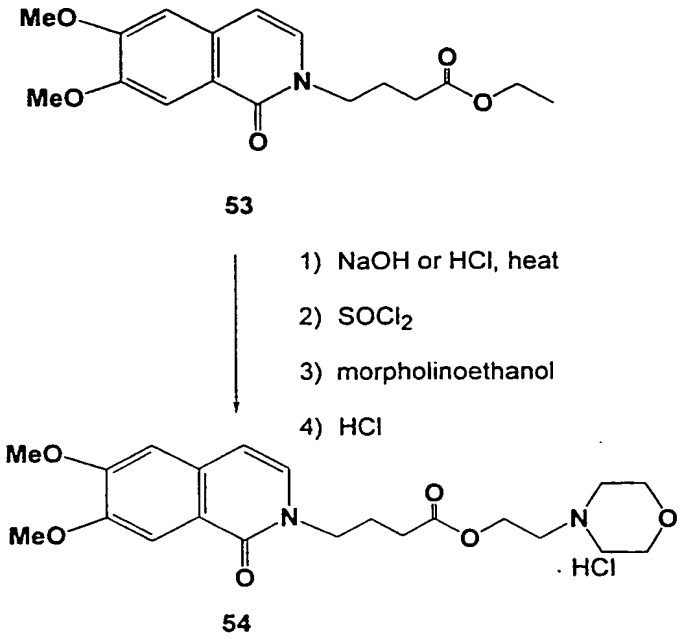
44. The compound according to Claim 43, wherein the bond is a tertiary N acyloxymethyl amide bond.

45. The compound according to Claim 39 mixed in a colloid.

46. The compound according to Claim 45, wherein the colloid is a liposome.

47. A pharmaceutical composition comprising the compound of Claim 39 and a pharmaceutically acceptable carrier.

48. A compound comprised of a first constituent having the formula:



further comprised of a second constituent conjugated to the first constitunt and consisting of a pyridinylimidazole which selectively antagonizes p38 activation.

49. The compound according to Claim 48, wherein the pyridinylimidazole is a cytokine suppressive anti-inflammatory drug.

50. The compound according to Claim 49, wherein the cytokine suppressive anti-inflammatory drug is selected from the group of such drugs comprising 1-[3-(4-morpholinyl)propyl]-4-(4-fluorophenyl-5-(4-pyridyl)imidazole, 2-(4-methylsulfinylphenyl)-3-(4-pyridyl)-6,7-dihydro-[5H]-pyrrolo[1,2-a]imidazole, 2-(4-methylthiophenyl)-3-(4-pyridyl)-6,7-dihydro-[5H]-pyrrolo[1,2-a]imidazole, 4-(4-fluorophenyl)-2-(4-methylsulfinyl)-5-(4-pyridyl)imidazole and 5-(4-pyridyl)-6(4-flurophenyl)-2,3-dihydroimidazole(2,1-b)thia zol.

51. The compound according to Claim 48, wherein the first and second constituents are conjugated to one another by a bond which is severable *in vivo*.

52. The compound according to Claim 51, wherein the bond is an amide, ester or azo bond.

53. The compound according to Claim 52, wherein the bond is a tertiary N acyloxymethyl amide bond.

54. The compound according to Claim 48 mixed in a colloid.

55. The compound according to Claim 54, wherein the colloid is a liposome.

56. A pharmaceutical composition comprising the compound of Claim 48 and a pharmaceutically acceptable carrier.

57. A method for treating a vertebrate host for an inflammatory condition comprising administering the compound of Claim 1 to the host.

58. The method according to Claim 57 wherein the inflammatory condition is arthritis, spondylitis, bone resorption, sepsis, septic shock, endotoxic shock, inflammatory bowel disease, asthma, bronchitis, chronic pulmonary inflammatory disease, silicosis, repurfusion injury, graft versus host reactions, allograft rejection, fever, viral and bacterial infection, inflammation of the joints, psoriasis and eczema, radiation dermatitis, multiple sclerosis, cell senescence and apoptosis.

59. A method for treating a vertebrate host for an inflammatory condition comprising administering the compound of Claim 24 to the host.

60. The method according to Claim 59 wherein the inflammatory condition is arthritis, spondylitis, bone resorption, sepsis, septic shock, endotoxic shock, inflammatory bowel disease, asthma, bronchitis, chronic pulmonary inflammatory disease, silicosis, repurfusion injury, graft versus host reactions, allograft rejection, fever, viral and bacterial infection, inflammation of the joints, psoriasis and eczema, radiation dermatitis, multiple sclerosis, cell senescence and apoptosis.

61. A method for treating a vertebrate host for an inflammatory condition comprising administering the compound of Claim 30 to the host.

62. The method according to Claim 61 wherein the inflammatory condition is arthritis, spondylitis, bone resorption, sepsis, septic shock, endotoxic shock, inflammatory bowel disease, asthma, bronchitis, chronic pulmonary inflammatory disease, silicosis, repurfusion injury, graft versus host reactions, allograft rejection, fever, viral and bacterial infection, inflammation of the joints, psoriasis and eczema, radiation dermatitis, multiple sclerosis, cell senescence and apoptosis.

63. A method for treating a vertebrate host for an inflammatory condition comprising administering the compound of Claim 39 to the host.

64. The method according to Claim 63 wherein the inflammatory condition is arthritis, spondylitis, bone resorption, sepsis, septic shock, endotoxic shock, inflammatory bowel disease, asthma, bronchitis, chronic pulmonary inflammatory disease, silicosis, repurfusion injury, graft versus host reactions, allograft rejection, fever, viral and bacterial infection, inflammation of the joints, psoriasis and eczema, radiation dermatitis, multipie sclerosis, cell senescence and apoptosis.

65. A method for treating a vertebrate host for an inflammatory condition comprising administering the compound of Claim 48 to the host.

66. The method according to Claim 65 wherein the inflammatory condition is arthritis, spondylitis, bone resorption, sepsis, septic shock, endotoxic shock, inflammatory bowel disease, asthma, bronchitis, chronic pulmonary inflammatory disease, silicosis, repurfusion injury, graft versus host reactions, allograft rejection, fever, viral and bacterial infection, inflammation of the joints, psoriasis and eczema, radiation dermatitis, multiple sclerosis, cell senescence and apoptosis.

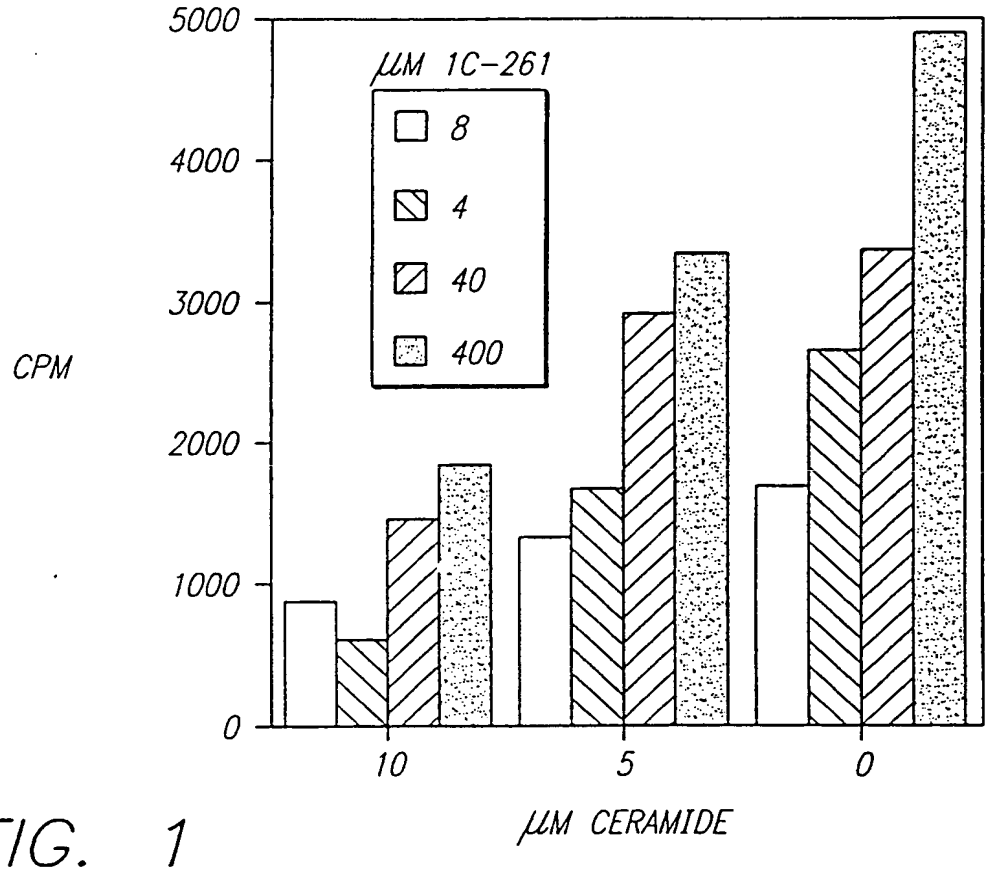


FIG. 1

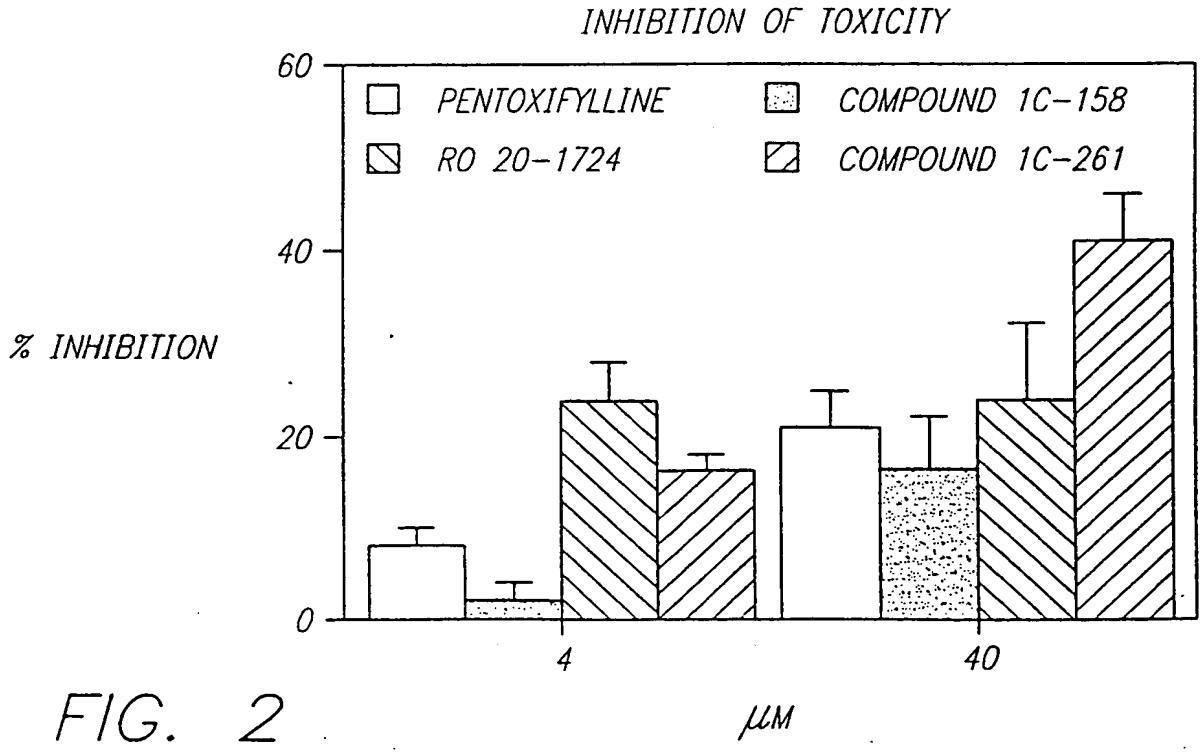


FIG. 2

FIG. 3

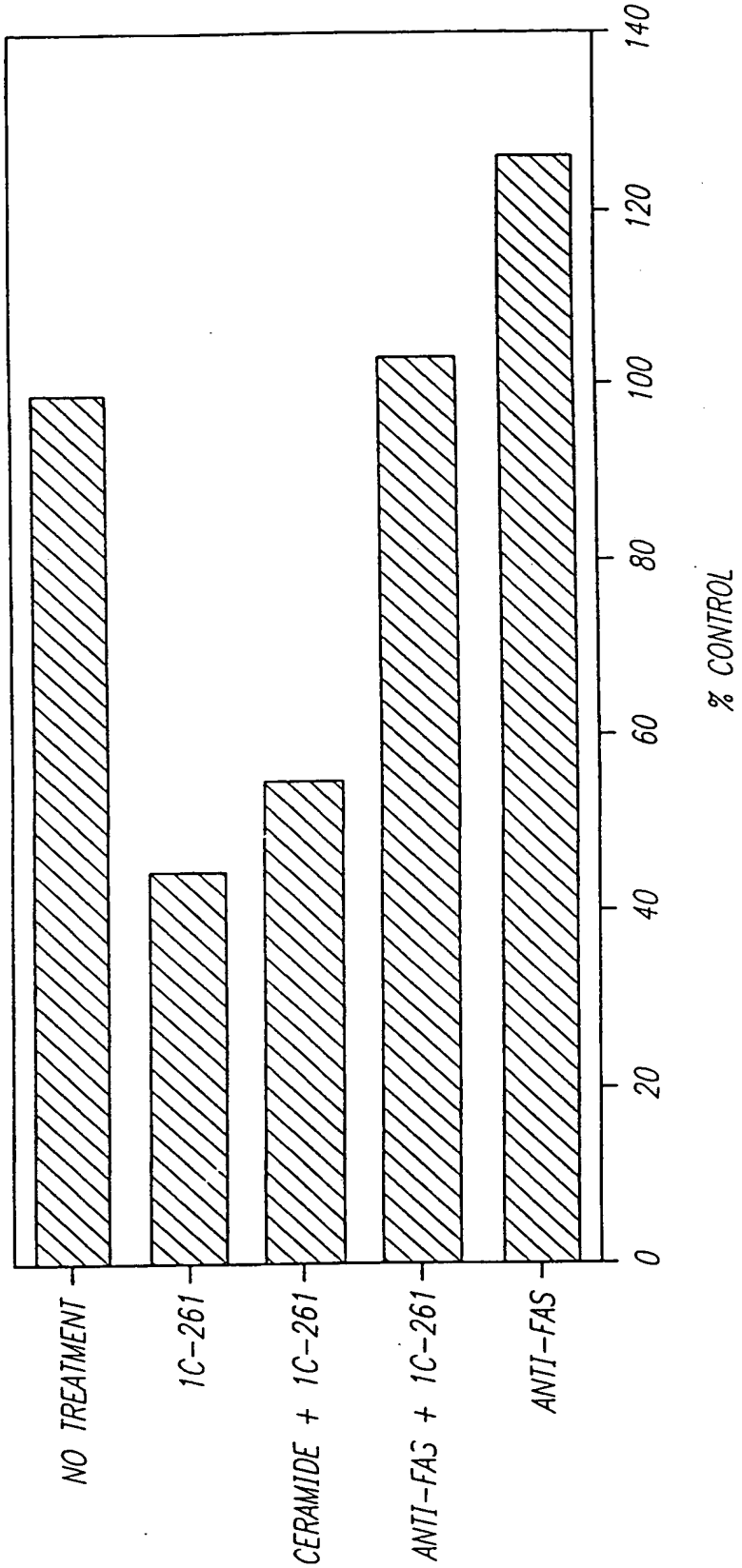


FIG. 4(A)

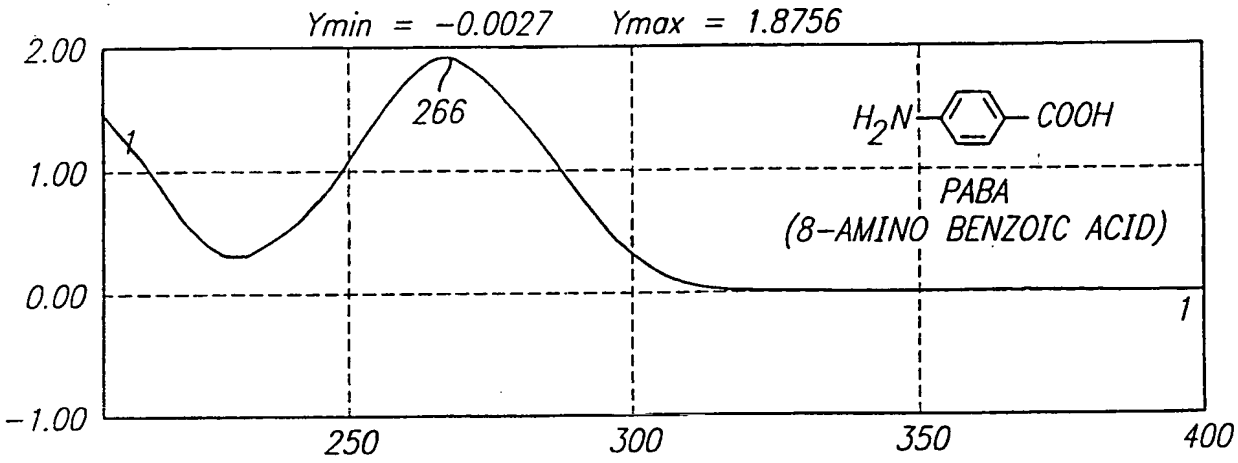


FIG. 4(B)

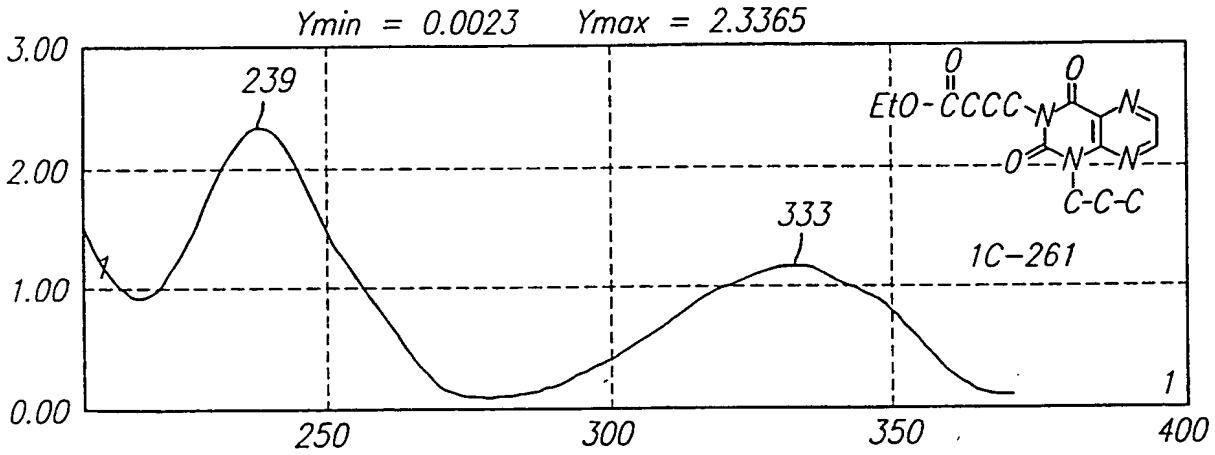


FIG. 4(C)

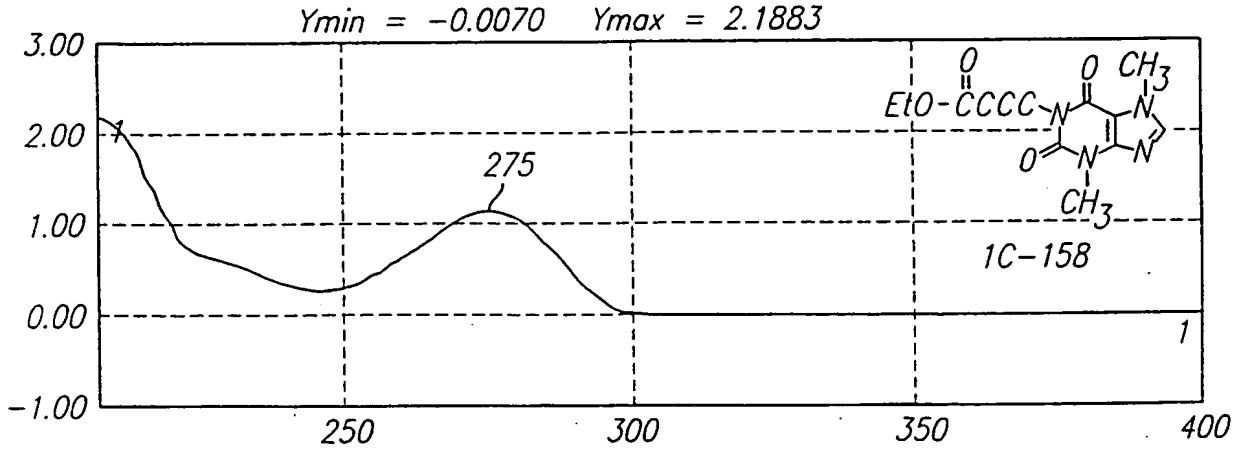


FIG. 4(D)

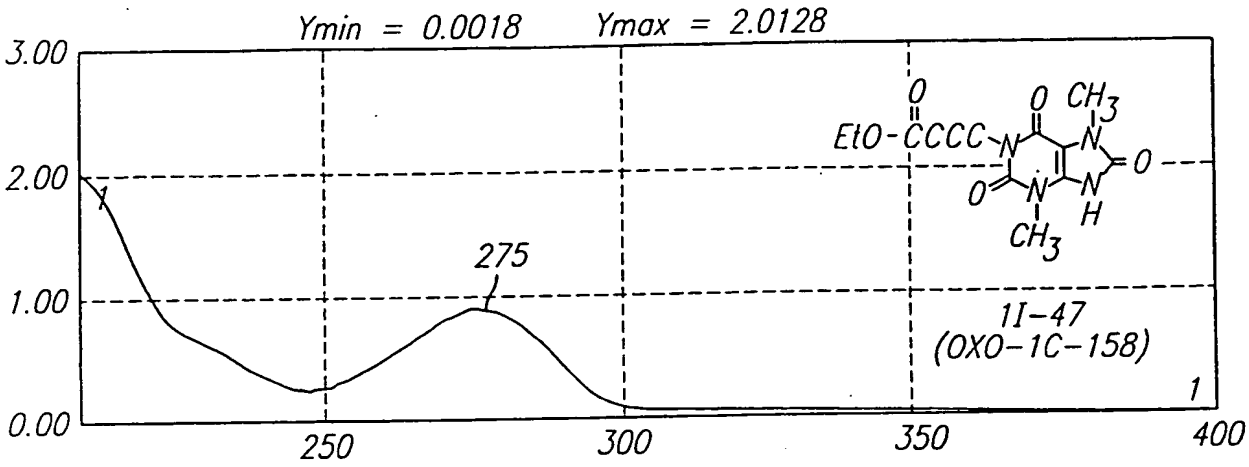


FIG. 4(E)

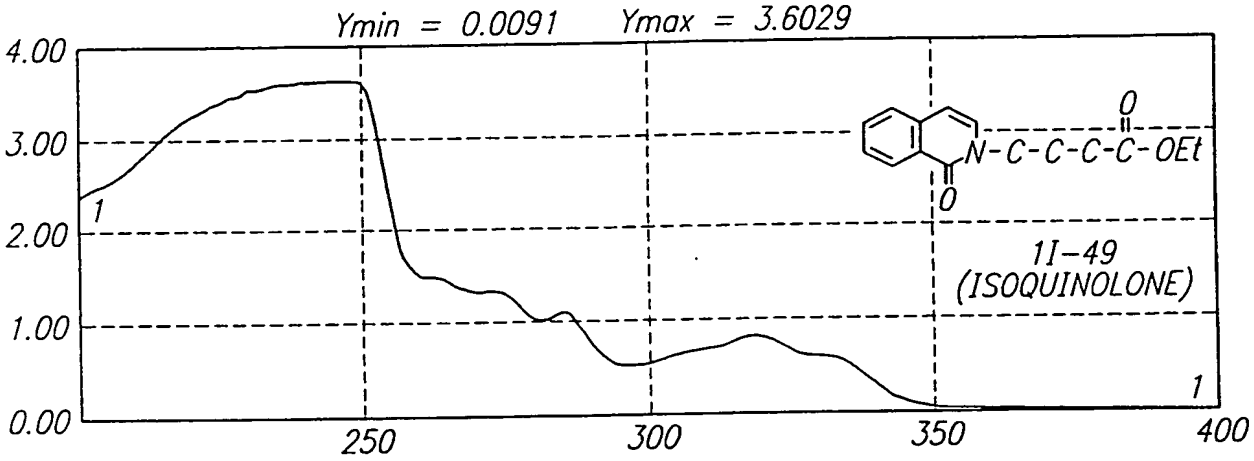


FIG. 4(F)

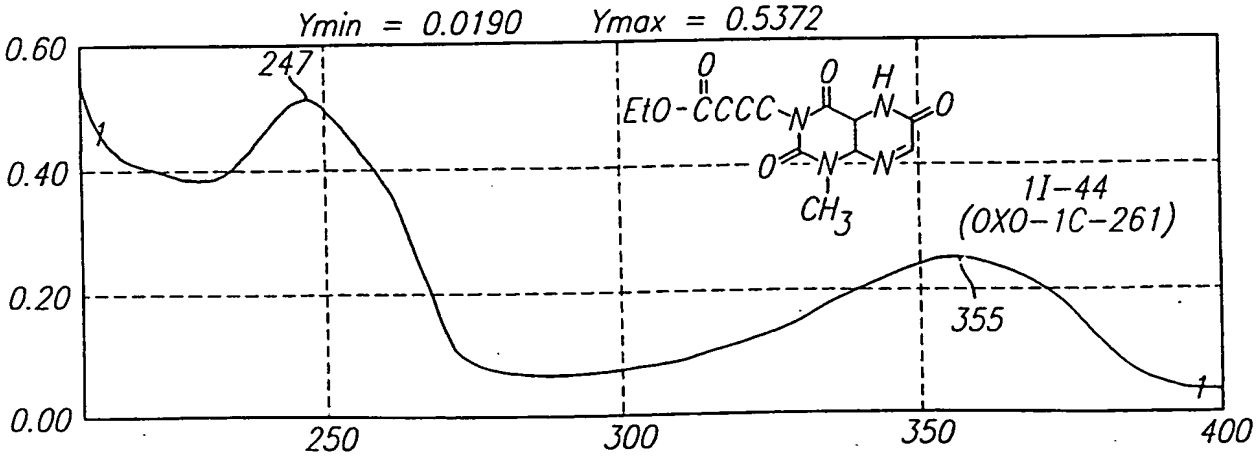


FIG. 4(G)

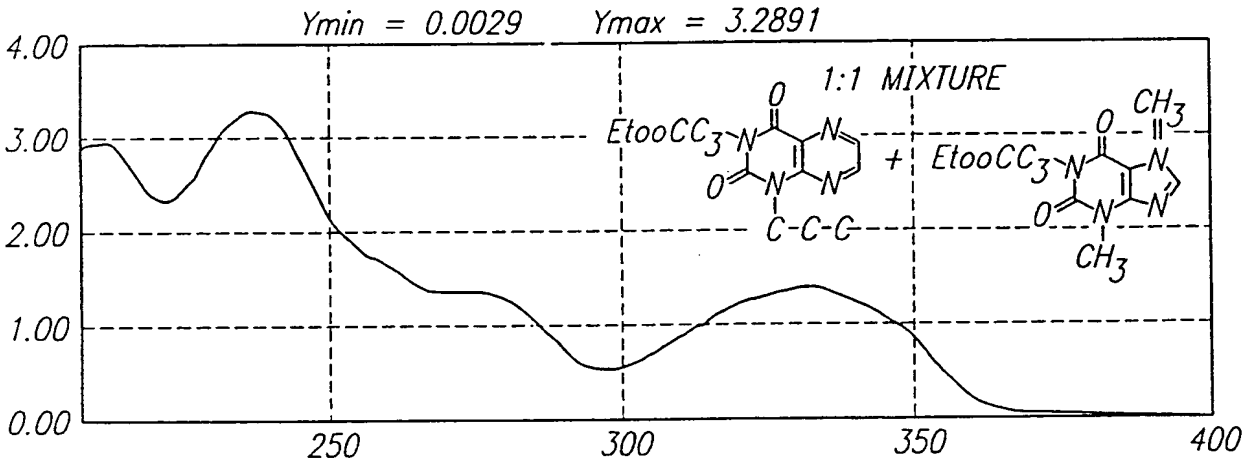


FIG. 5(b)

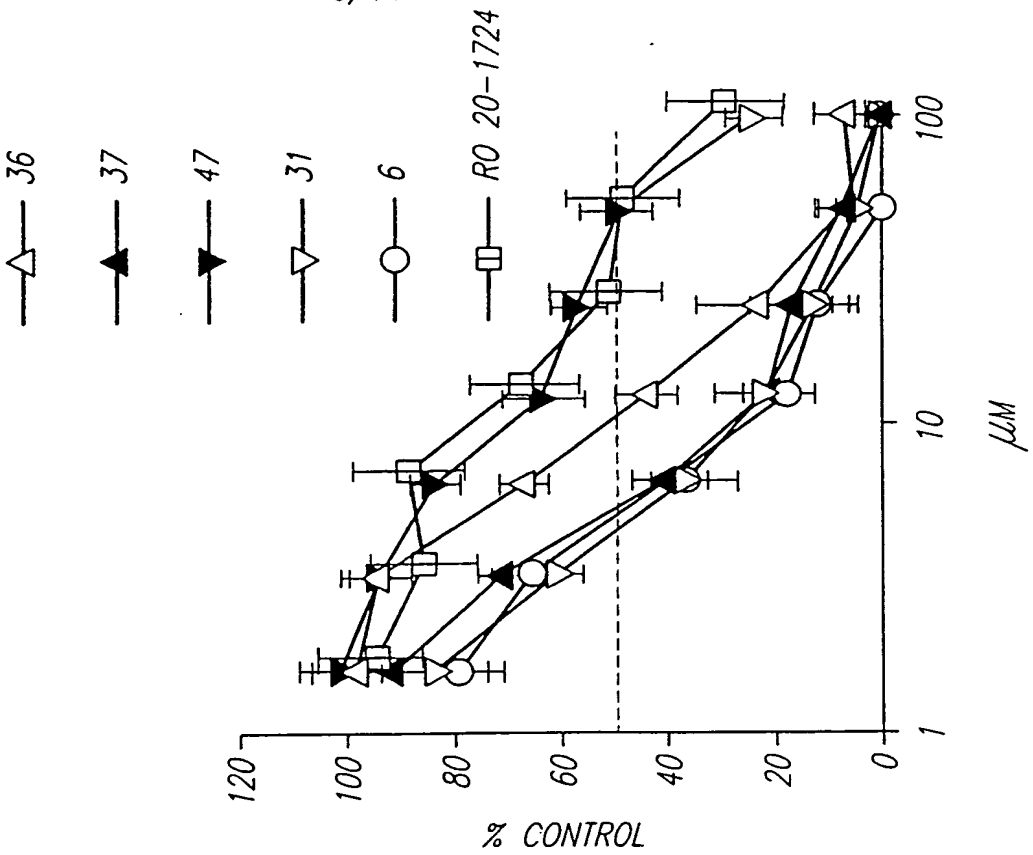


FIG. 5(a)

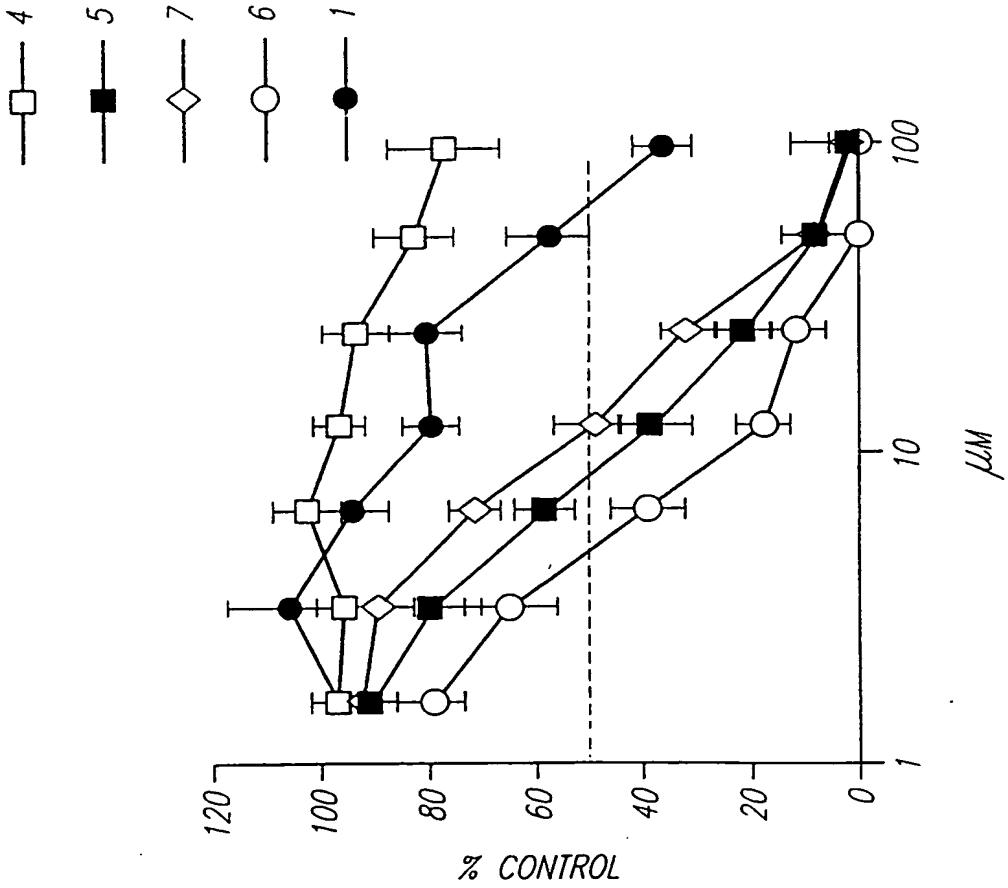


FIG. 6

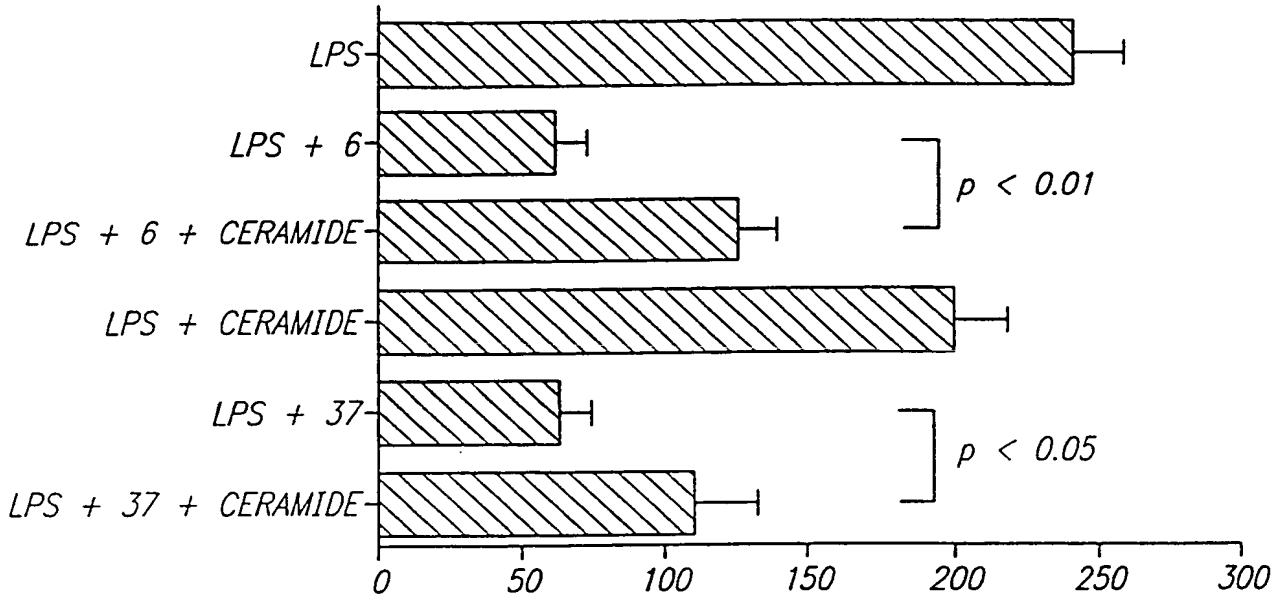
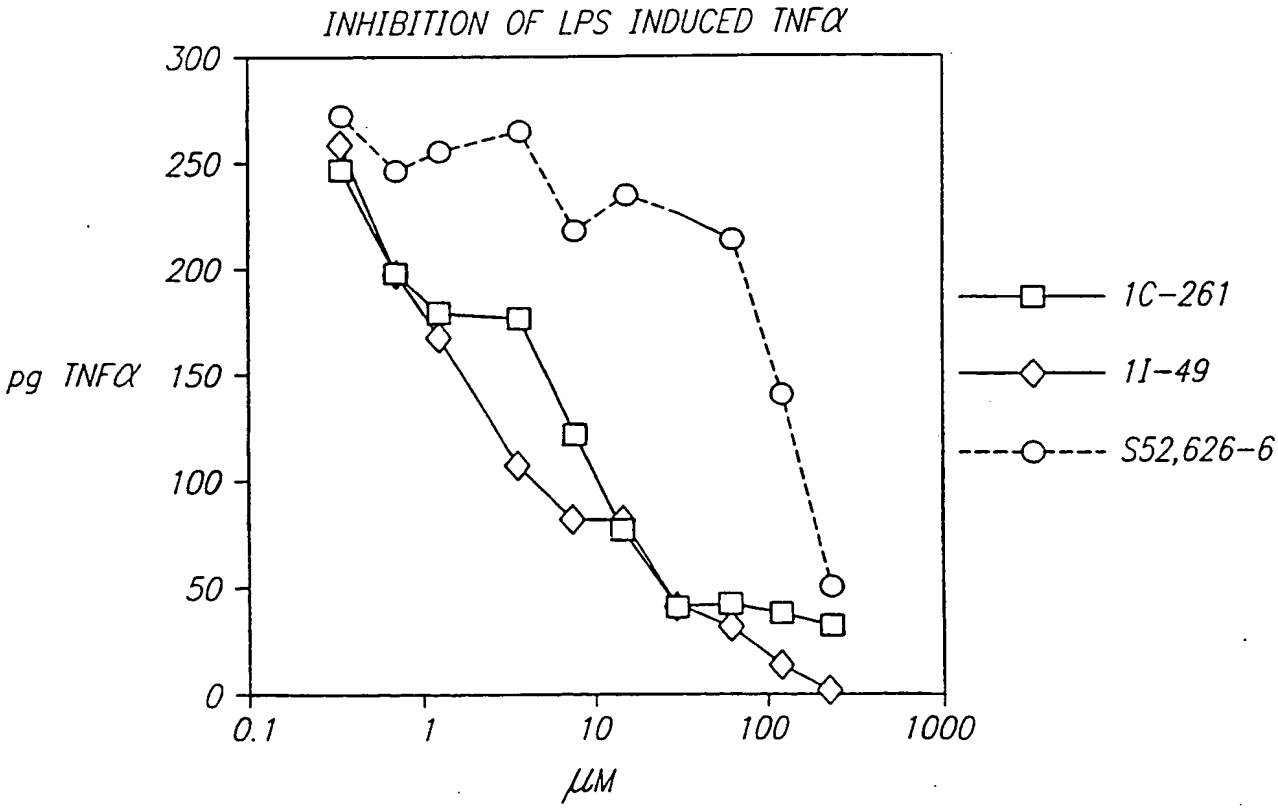


FIG. 9



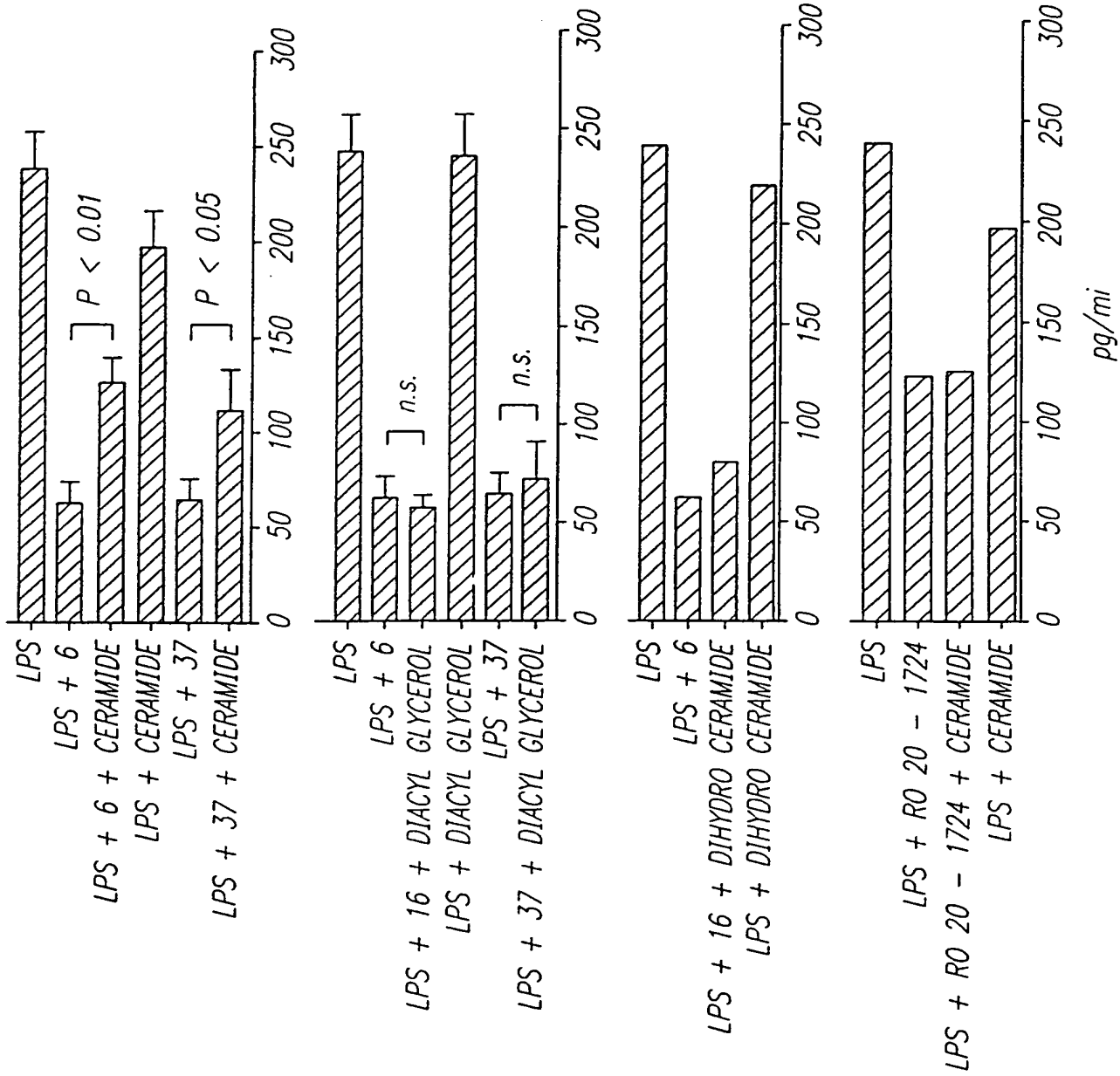


FIG. 7

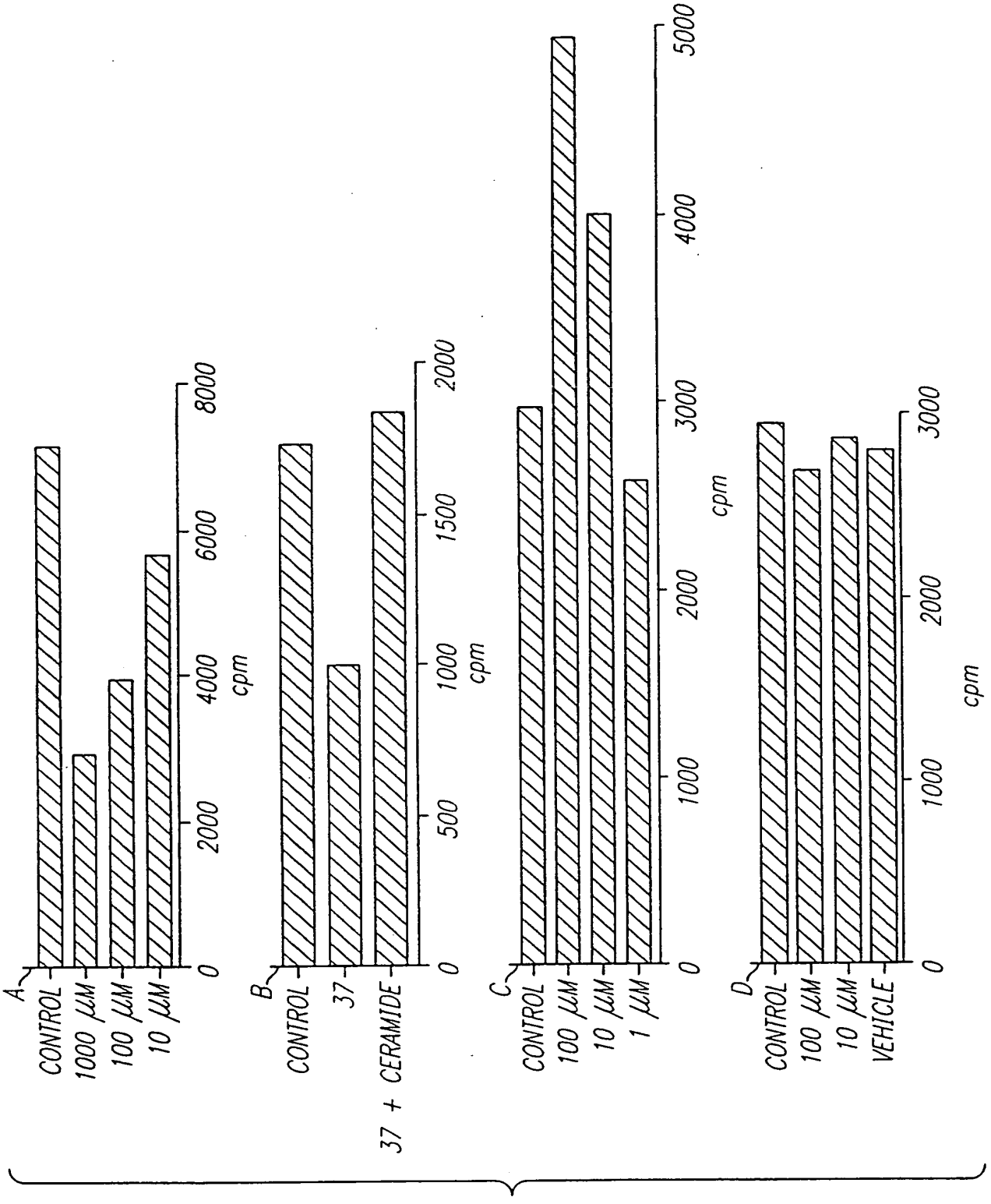


FIG. 8

FIG. 10

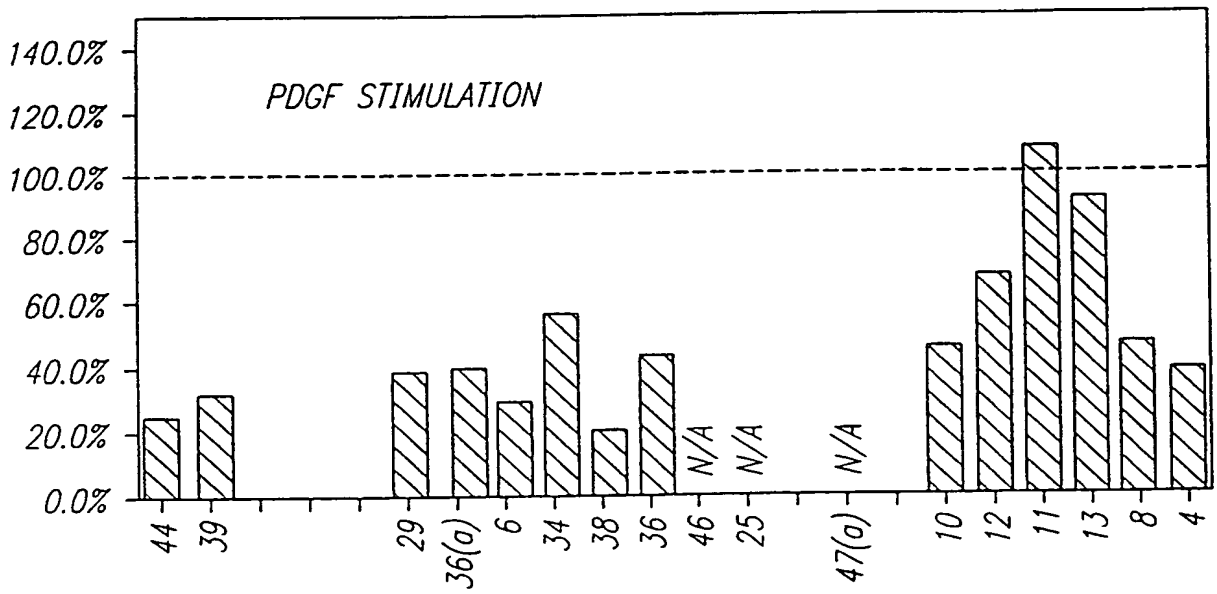


FIG. 11

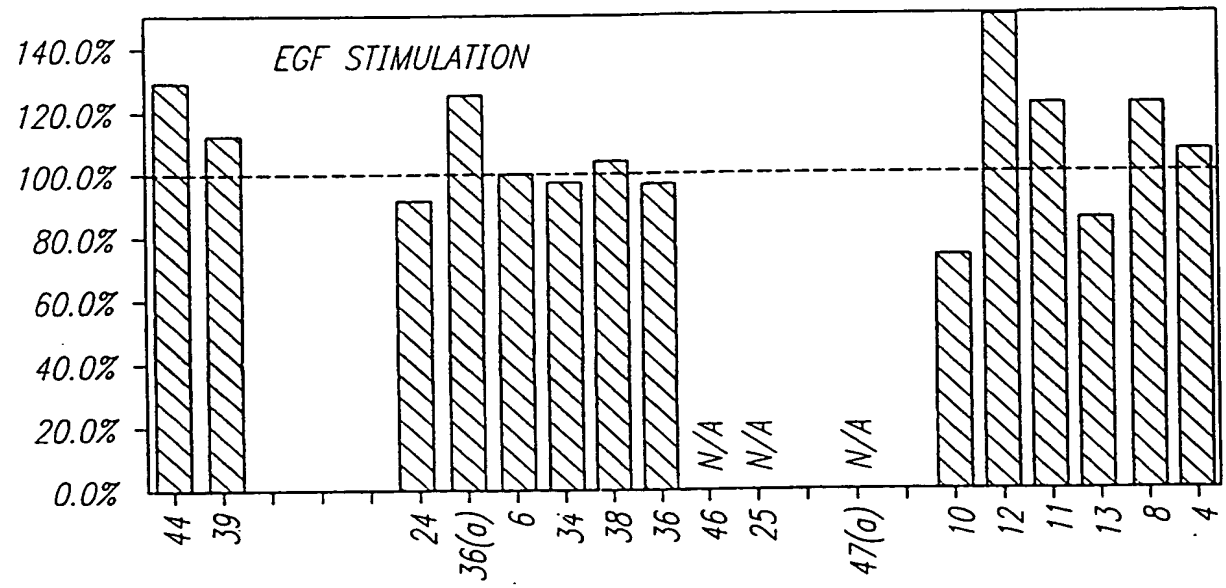


FIG. 12

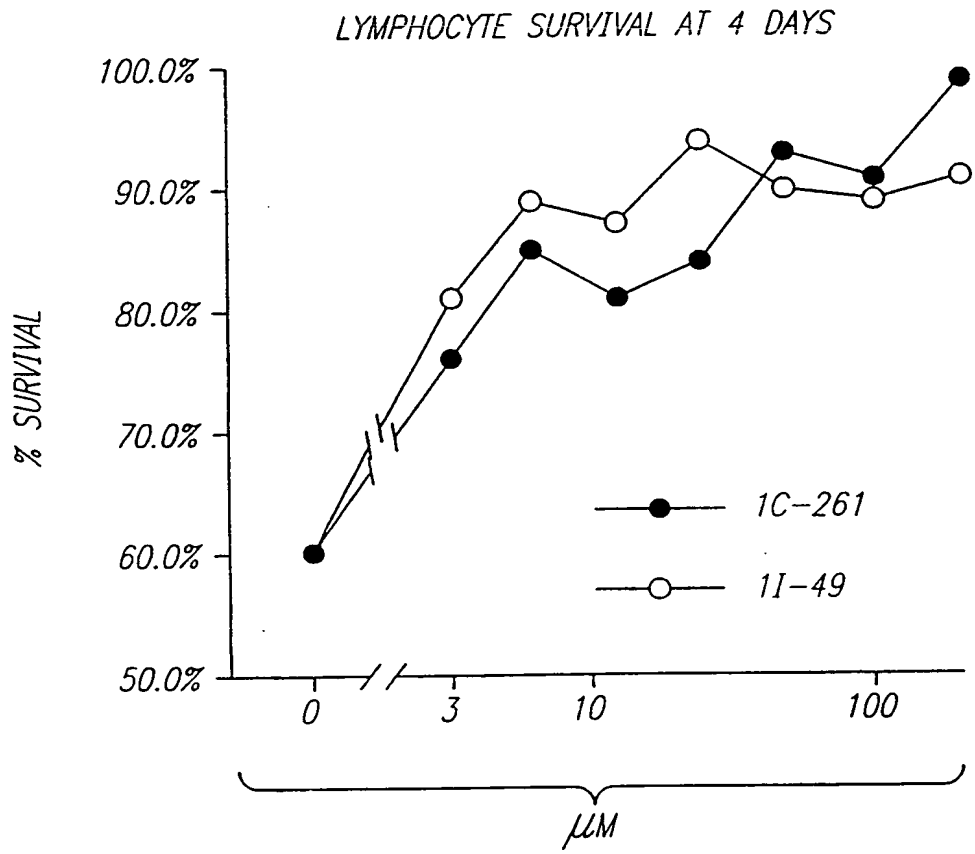
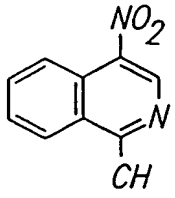
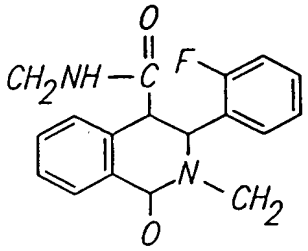


FIG. 13(A)

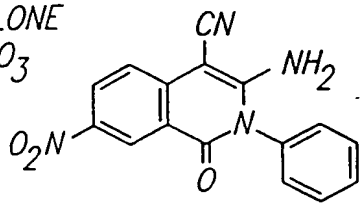
S45,928-3
3-NIITROIXOCARBOSTYRIL
$C_9H_6N_2O_3$



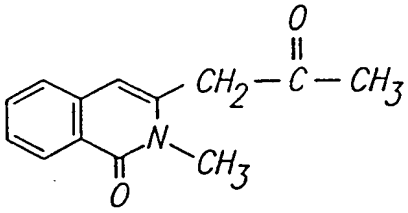
S76,334-9
N2DIMMETHYL-3-(3-FLUOROPHENYL)-1-OXO-1234
TETRAHYDRO-4-ISOQUINOLINECARBOXAMIDE
$C_{18}H_{17}FN_2O_2$



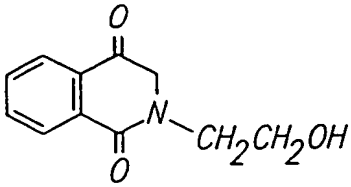
S93,63C-8
3-AMINO-CYANO-7-NITRO-2-PHENYL-1(2H)-
ISOQUINOLONE
$C_{15}H_{10}N_4O_3$



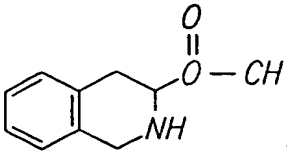
S75,469-8
2-METHYL-3-(2-OXOPROPYL)-1(2H)_ISOQUINOLINE
CNE
$C_{13}H_{13}NO_2$



S88,370-0
2-(2-HYDROXYETHYL)-1,4-(2H,3H)-ISOQUINOLONE
$C_{11}H_{11}NO_3$



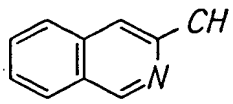
21,493-0
(H)-1234 TETRAHYDRO-3-ISOQUINOLINE
CARBOXYLIC ACID HYDROCHLORIDE
$C_{10}H_{11}NO_2$



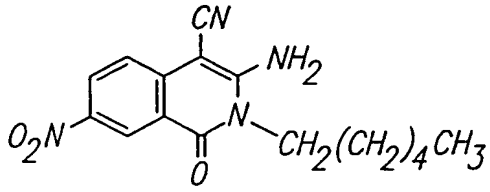
*HC

FIG. 13(B)

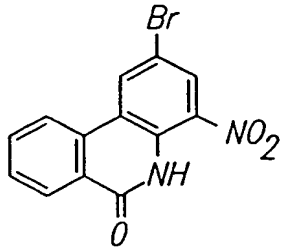
36,895-4
3-HYDROXYISOQUINOLINE,99%
C_9N_7NO



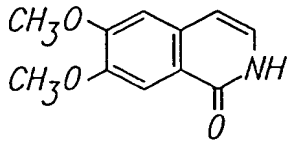
S94,009-7
3-AMINO-4-CYANO-2-HEXYL-7-NITRO-1(2H)-IOSQUINOLONE
$C_{16}H_{18}N_4O_3$



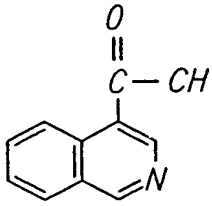
S75,699-7
2-BROMO-4-NITRO-6(5H)-PHENANTHRIDINONE
$C_{13}H_7BrN_2O_3$



S52,626-6
6,7-DIMETHOXY-1(2H)-ISOQUINOLONE
$C_{11}H_{11}NO_3$



S84,100-5
4-ISOQUINOLINECARBOXYLIC ACID
$C_{10}H_7NO_2$



15,013-4
1-ISOQUINOLINECARBOXYLIC ACID, 99%
$C_{10}H_7NO_2$

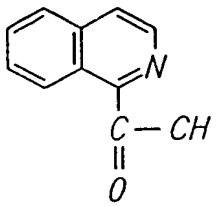
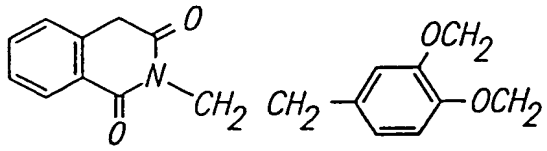
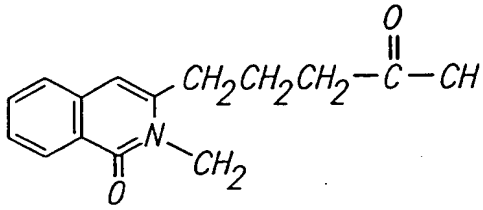


FIG. 13(C)

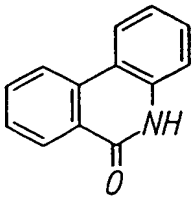
S76,087-0
2-(2-(3,4-DIMETHOXYPHENYL)ETHYL)ISOQUINOLINE-1,3(2H,4H)-DIONE
$C_{19}H_{19}NO_4$



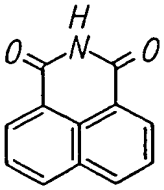
S76,473-6
1,2-DIHYDRO-2-METHYL-1-OXO-3-ISOQUINOLINES UTYRIC ACID
$C_{14}H_{15}NO_3$



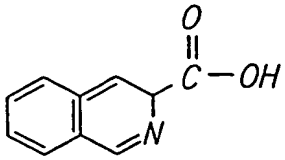
29,963-4
5(5H)-PHENANTHRIDINONE
$C_{13}H_9NO$



N165-8
1,8-NAPHTHALIMIDE, 99%
$C_{12}H_7NO_2$



33,854-0
3-ISOQUINOLINECARBOXYLIC ACID HYDRATE, 99%
$C_{10}H_7NO_2$



$*xH_2O$

INTERNATIONAL SEARCH REPORT

Interr nal Application No
PCT/US 99/14320

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07D475/02 C07D473/04 C07D513/04 C07D239/96 C07D217/24
C07D401/14 A61K31/505 A61K31/52 //(C07D513/04,285:00,
239:00)
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED
Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07D A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 96 20710 A (UNIV CALIFORNIA) 11 July 1996 (1996-07-11) cited in the application claims	1-66
Y	WO 94 22449 A (CELL THERAPEUTICS INC) 13 October 1994 (1994-10-13) claims	1-66
A	WO 94 22863 A (CELL THERAPEUTICS INC) 13 October 1994 (1994-10-13) claims	1-66
A	WO 92 21344 A (HUTCHINSON FRED CANCER RES) 10 December 1992 (1992-12-10) claims	1-66

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
15 October 1999	22/10/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Chouly, J

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Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Inter- ınal Application No
PCT/US 99/14320

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
P,Y	WO 98 52948 A (UNIV CALIFORNIA) 26 November 1998 (1998-11-26) cited in the application claims 1-66	1-66

1

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Ir ational application No.
PCT/US 99/ 14320

Box I Observations wher certain claims were found uns archable (Continuation f item 1 of first she t)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 57-66
because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claims 57-66 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1 [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2 [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3 [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4 [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

ormation on patent family members

Interr ıal Application No
PCT/US 99/14320

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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